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THE EFFECT OF CERTAIN HORMONES
ON TISSUE RESPIRATION IN THE RAT

A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES
IN PARTIAL FULFILMENT OF THE REQUIREMENTS
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by

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ABSTRACT

An investigation was undertaken to determine the effects of altered thyroid states on the respiration of diaphragm, heart and brain of the rat. Normal and thyroidectomized male rats were given L-triiodothyronine and the QO_2 values for each of the tissues were determined. The effect on tissue respiration of a special diet, low in iodine and deficient in animal protein, was also studied. Studies on the effects of adrenalectomy, thyroidectomy, and combined thyroidectomy and adrenalectomy were carried out. The effects of hypophysectomy and administration of TSH on respiration were investigated. Oxygen consumption of the tissues was measured in Huston-Martin flasks by the direct method of Warburg.

Thyroidectomy depressed the respiration of diaphragm, heart and brain except in Series I. Administration of L-triiodothyronine to normal rats increased the respiration of diaphragm; the increase was significant when the rats were injected 4 days before sacrificing. Treatment of thyroidectomized rats with L-triiodothyronine increased the respiration significantly in all cases.

Administration of L-triiodothyronine to normal rats increased the respiration of the heart but not significantly. Treatment of thyroidectomized rats with L-triiodothyronine increased the respiration of the heart in all cases; the increase was significant in the 7 day rats.

The special diet did not appear to affect the respiration of the diaphragm and heart appreciably.

Adrenalectomy depressed the respiration of all tissues; the respiration was significantly below that of normal in diaphragm and heart. Combined thyroidectomy and adrenalectomy depressed the respiration of all tissues significantly below that of normal. The combined surgery significantly depressed the respiration of all tissues below that of tissues from adrenalectomized animals but not below that of tissues from thyroidectomized animals.

Hypophysectomy depressed the respiration of diaphragm, heart and brain. Administration of TSH to normal animals 4 hours before sacrificing increased the respiration of all tissues investigated. The administration of TSH to normal animals 24 hours before sacrificing and to hypophysectomized animals 4 hours before sacrificing caused an increase in the respiration of the diaphragm, a decrease in the respiration of the heart, and a slight increase in the respiration of the brain. Similar results were obtained when TSH was administered to hypophysectomized rats 24 hours before sacrificing with the exception of brain respiration which was depressed.

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TABLE OF CONTENTS

	Page
Abstract-----	i
Acknowledgements-----	iii
List of Tables-----	vii
List of Figures-----	viii
I. INTRODUCTION-----	1
II. SURVEY OF THE LITERATURE-----	3
A. Physiology of the Thyroid Gland-----	3
1. Early Historical Developments-----	3
2. Synthesis of Thyroid Hormone-----	4
3. Storage and Release of Hormone-----	6
4. Alteration of the Thyroxine Molecule by Peripheral Tissues-----	7
5. Pituitary Thyroid Relationships-----	7
B. Chemistry and Pharmacology of Drugs Used in this Investigation-----	8
1. Triiodothyronine-----	8
2. Thyrotropic Hormone-----	10
C. The Effects of Thyroid Hormones on the Respiration of Tissue-----	11
1. In Vitro Studies-----	11
2. In Vivo Studies-----	12

	Page
a. Diaphragm-----	12
b. Heart-----	13
c. Brain-----	15
3. Studies on Adrenal Thyroid	
Interrelationships-----	16
4. The Effect of Hypophysectomy and TSH	
Administration on Oxygen Consumption-----	18
III. STATEMENT OF THE PROBLEM-----	20
IV. EXPERIMENTAL-----	21
1. Method of Handling the Animals and Drugs-----	21
2. Tissue Respiration Apparatus-----	24
3. Method of Tissue Respiration-----	25
4. Calculation of Results-----	25
V. RESULTS-----	27
1. Effect of L-triiodothyronine on Tissue	
Respiration of Diaphragm and Heart-----	27
2. Effects of a Special Diet on Tissue	
Respiration of Diaphragm and Heart-----	28
3. Effect of L-triiodothyronine on	
Brain Respiration-----	30
4. Effect of Thyroidectomy, Adrenalectomy,	
and Combined Thyroidectomy and Adrenalectomy	
on Tissue Respiration-----	30
5. Effect of Hypophysectomy and Thyrotropin	
on Tissue Respiration-----	34

	Page
6. Comparison of the Results when the Mean QO_2 Values are Expressed as a Percentage of Their Controls-----	36
VI. DISCUSSION-----	44
1. General Discussion of Tissue Respiration-----	44
2. Tissue Respiration of Normal Animals-----	46
3. Effect of Thyroidectomy on the Respiration of Diaphragm and Heart-----	47
4. Effect of Triiodothyronine on Tissue Respiration-----	48
a. Diaphragm-----	48
b. Heart-----	48
5. Effect of the Special Diet on Tissue Respiration-----	49
6. Effect of Triiodothyronine on the Respiration of the Brain-----	50
7. Effect of Thyroidectomy, Adrenalectomy, and Combined Thyroidectomy and Adrenalectomy on Tissue Respiration-----	50
8. Effect of Hypophysectomy and Thyrotropin on Tissue Respiration-----	52
VII. SUMMARY AND CONCLUSIONS-----	54
BIBLIOGRAPHY-----	56
APPENDIX following page _____	61

LIST OF TABLES

Table	Page
I Effect of L-triiodothyronine on the Respiration of Diaphragm and Heart-----	29
II Effect of L-triiodothyronine on the Respiration of Brain Slices-----	31
III Effect of Adrenalectomy, Thyroidectomy, and Combined Thyroidectomy and Adrenalectomy on the Respiration of Diaphragm, Heart, and Brain-----	32
IV Effect of Hypophysectomy and Thyrotropin on the Respiration of Diaphragm, Heart, and Brain-----	35
V Summary of Mean QO_2 Values Expressed as a Percentage of Controls-----	37
VI Summary of Mean QO_2 Values Expressed as a Percentage of Normal-----	38
VII Summary of Mean QO_2 Values Expressed as a Percentage of Controls-----	39

LIST OF FIGURES

Figure		Page
I	Effect of L-triiodothyronine on Tissue Respiration in the Rat-----	40
II	Effect of Thyroidectomy, Adrenalectomy, and Combined Thyroidectomy and Adrenalectomy on Tissue Respiration in the Rat-----	41

I INTRODUCTION

The thyroid gland, first described in 1656 by Wharton, is not essential to life but normal physiologic function depends very much upon its hormonal elaboration. Abnormal function of the gland affects basal metabolic rate, cardiac function, growth, the nervous system, and mentality.

Until the time of the isolation of thyroxine and its final chemical identification, the thyroid gland had occupied a central position in endocrine research. After this time it appeared for the moment as if biochemical work on the thyroid had come to an end. Many factors have played a role in the revival of interest in the thyroid gland. The development of knowledge of the biosynthesis of thyroxine, the successful application of I^{131} to research on the physiology of the thyroid and to therapy of disease and the discovery of goitrogens have all played a part in creating renewed interest. The discovery by Gross and Pitt-Rivers and independently by Roche and Michel, of triiodothyronine in the thyroid gland and in the blood plasma and the observation of Pitt-Rivers and her colleagues that triiodothyronine is considerably more active than thyroxine has also stimulated new interest in thyroid research.

Although knowledge of the thyroid and its function has grown tremendously since Wharton first described it there are still many basic questions left unanswered. One of the most challenging problems is the

identification of the active thyroid hormone which acts at the cellular level. The mechanism of action of the thyroid hormone is still to be discovered and the mechanism by which thyroid hormone release is controlled is still uncertain.

It was hoped that by tissue studies, using the Huston-Martin technique, additional facts could be obtained to eventually discover the answers to some of the many questions still confronting physiologists investigating the thyroid.

II SURVEY OF THE LITERATURE

A. Physiology of the Thyroid Gland

1. Early Historical Developments

In 1835 Robert J. Graves (1) reported the symptoms of a syndrome which later became known as Grave's disease. Gull (2) described before the Clinical Society of London in 1874 "a cretinoid condition supervening in adult life in women." Ord (3) suggested that the malady be called myxedema. The demonstration by Kocher (4) that this syndrome developed following thyroidectomy and the report by Murray (5) in 1891 that myxedematous patients were restored to normal health by administration of thyroid extract gave adequate proof that the thyroid elaborates a substance that is necessary to normal health.

These classic human experiments excited a series of investigations into the thyroid hormone, the factors which control its synthesis and secretion, and its mode of action.

The first clue of the nature of the thyroid hormone came when Baumann (6) in 1885 demonstrated that the thyroid was rich in iodine and subsequently that the iodine in the thyroid exists mainly as thyroglobulin. The extensive work of Ostwald and that of Hutchison (7) on thyroglobulin led eventually to the isolation of thyroxine by Kendall in 1915 (8).

Harington and co-workers (9) not only isolated thyroxine and diiodotyrosine but also synthesized both of these iodinated compounds and demonstrated that thyroxine has physiological activity.

2. Synthesis of Thyroid Hormone

It is generally agreed that the synthesis of the thyroid hormone occurs by the following steps.

- a. Concentration of iodide in the thyroid.
- b. Oxidation of iodide to iodine and iodination of tyrosine to diiodotyrosine.
- c. Coupling of two molecules of diiodotyrosine to give thyroxine.
- d. The formation of triiodothyronine.

Each of these steps will be considered separately.

a. The Iodide-Concentrating Mechanism of the Thyroid

Iodine present in the blood plasma in small amounts as inorganic iodide is concentrated in the acinar cells of the thyroid. The gland has remarkable powers of accumulating iodide as can be readily demonstrated with the aid of I^{131} , and this accumulation is accelerated by thyrotrophic hormone (10) and is inhibited by thiocyanate (11). Normally the iodide becomes organically bound as fast as it is accumulated but, after administration of anti-thyroid agents such as thiourea, the organic combination is blocked and a high concentration of inorganic iodide remains in the gland.

b. Conversion of Iodide to Iodine and Iodination of Tyrosine to Diiodotyrosine

Before the iodide which is concentrated by the thyroid can react with tyrosine, it must be oxidized to iodine. This process can be due to various oxidizing enzymes, none of which have been isolated or characterized as specific. Dempsey (12) (13) however has demonstrated both oxidase and peroxidase activity in the gland. De Robertis and Grasso (14) have confirmed the finding of peroxidase.

The iodine never appears as such in the free state but is immediately taken up by the tyrosine of the glandular protein to form monoiodotyrosine and diiodotyrosine.

c. Biosynthesis of Thyroxine

Strong support for the thesis, originally advanced by Harington, that in the thyroid the synthesis of thyroxine occurs as the result of some coupling of two diiodotyrosine molecules came from early studies done with radioactive iodine by Mann, Leblond, and Warren (15) and by Perlman, Morton, and Chaikoff (16). These workers followed the fate of radioactive iodine in the thyroids of rats at varying intervals after administering carrier-free tracers of I^{131} . They found radioactive iodide, diiodotyrosine, and thyroxine in the thyroids of their animals. Diiodotyrosine was present in the greatest concentration. However, the longer the time interval after administering the I^{131} , the greater was the amount of I^{131} found in the thyroxine fraction. Subsequent studies (17) (18) in which chromatographic methods have been used in separating the various compounds of the thyroid have also revealed significant amounts of monoiodotyrosine and small amounts of triiodothyronine in the thyroids of experimental animals.

d. Biosynthesis of Triiodothyronine

With regard to the biosynthesis of triiodothyronine, two pathways are

possible: (1) enzymic reduction of one iodine atom from thyroxine which was first favored by Gross and Pitt-Rivers (19) and (2) the coupling of one molecule of monoiodotyrosine with one molecule of diiodotyrosine. Roche et al. (20) (21) were unable to demonstrate any action of thyroid desiodinase on thyroxine or triiodothyronine. They concluded, therefore, that triiodothyronine could not arise in the thyroid gland by partial deiodination of thyroxine but must be formed by the coupling of one molecule of diiodotyrosine with one molecule of monoiodotyrosine. Feuer (22) by studying the formation of thyroid hormones in vivo found that the radioactivity of triiodothyronine initially exceeded that of thyroxine later becoming much less. For this reason he feels strongly that triiodothyronine is a precursor of thyroxine.

3. Storage and Release of Hormone

For a varying period of time after biosynthesis, the iodothyronines remain within the follicular colloid as part of the thyroglobulin molecule. The phase of storage is terminated by the proteolysis of thyroglobulin into its component peptides and amino acids.

The principle elaborations of the thyroid gland, triiodothyronine and thyroxine, leave the gland by diffusion and in the case of thyroxine, as a result of competitive surface-binding favoring the serum thyroxine-binding globulin (TBG) over thyroglobulin (23).

During zonal electrophoresis in several alkaline buffers, thyroxine is bound reversibly to three moieties within the extracellular fluid: thyroxine-binding globulin (TBG), thyroxine-binding pre-albumin (TBPA),

and serum albumin (24). In physiological concentrations the hormone is distributed almost equally between TBG and TBPA, and a minute fraction is bound to albumin. Triiodothyronine, less firmly bound to TBG than thyroxine, binds not at all to TBPA. How thyroxine is exchanged from extracellular binding components to intracellular sites of metabolic degradation or utilization is unknown and various hypotheses exist as to the mechanism of the exchange.

4. Alteration of the Thyroxine Molecule by Peripheral Tissues

For years one of the most challenging problems in thyroid physiology has been to determine what is the active peripheral thyroid hormone. Thyroglobulin was first thought to be the hormone, thyroxine was then suggested, and more recently triiodothyronine was thought to be the hormone which acted at the cellular level. Many in vivo and in vitro studies of the metabolism of thyroxine and triiodothyronine have added conceptual complexity to an understanding of the nature of the thyroid hormone by demonstrating that triiodothyronine, rather than occupying a central physiological role in the metabolism of thyroxine, is in fact a way-station along pathways of further molecular alteration of the parent molecule. Triiodothyronine is further deiodinated to diiodothyronine which is rapidly deiodinated. The physiological significance of the deiodination of thyroxine beyond triiodothyronine and of the degradation of the thyronine nucleus remains uncertain (25).

5. Pituitary Thyroid Relationships

In 1954 D'Angelo (26) stated the following: "The interplay between

anterior pituitary and the thyroid, considered in its simplest terms, is conceived to operate as follows: failure of the thyroid gland to secrete sufficient hormones for bodily requirements elicits unrestrained secretion of TSH from the pituitary, and the rising blood levels of thyrotropin stimulate the lagging thyroid to greater activity. Conversely, blood thyroid hormone levels in excess of the metabolic requirements act back on the pituitary to inhibit TSH secretion, as a consequence of which the thyroid reverts to its normal morphologic and physiologic state. The balanced relationship between the anterior pituitary and the thyroid thus is a servo-mechanism in which the circulating level of each respective hormone controls the output of the opposing hormone at its source."

D'Angelo realized this was an oversimplification of the events which actually occur. It is the feeling of most workers that there is an involvement with the hypothalamus which must be considered in this regard. D'Angelo found that thyroid function in general is impaired by lesions of the anterior hypothalamus (27).

B. Chemistry and Pharmacology of Drugs Used in this Investigation

1. Triiodothyronine

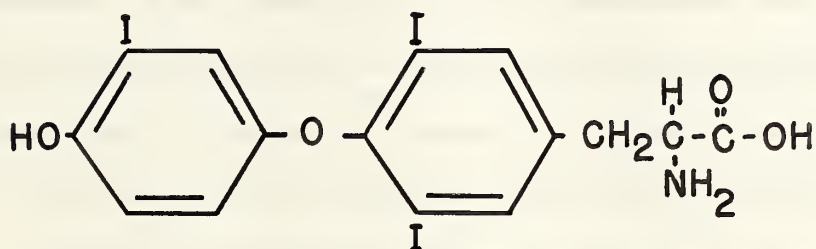
a. Isolation of Triiodothyronine

By the use of I^{131} and two dimensional chromatography Gross, Leblond, and colleagues (28) (29) (30) were able to identify in the blood of rats a compound which they called unknown I. This compound had an R_F value identical with that of thyroxine. A number of derivatives of thyroxine were compared with unknown I and it was found that triiodothyronine

and unknown I had identical R_F values. Isolation of triiodothyronine from the thyroid confirmed the identification of this compound as a thyroid hormone. While this work was in progress, Roche et al. (31) (32) simultaneously reported the synthesis of 3:5:3-triiodothyronine from 3:5-diiodothyronine and its chromatographic detection in rat thyroid hydrolysates after administration of I^{131} .

b. Chemistry and Pharmacology of L-triiodothyronine

The structural formula for sodium L-triiodothyronine may be represented as



L-triiodothyronine is approximately twice as active as the racemic form. The drug differs in chemical structure from thyroxine only in the absence of one iodine atom at position 5. It produces all of the qualitative metabolic and clinical effects of desiccated thyroid and thyroxine but differs quantitatively in being much more rapid in action with a shorter effect (33) and it is about 3-5 times more potent than an equal amount of L-thyroxine (34). L-triiodothyronine has been isolated in minute quantities from the thyroid gland and the blood plasma; however it is prepared synthetically

for commercial use. It is marketed as Cytomel by Smith, Kline and French. L-triiodothyronine is absorbed readily from the gastrointestinal tract. It is rapidly cleared from the blood stream and is more loosely bound to plasma proteins than is thyroxine. Little is known about the metabolism of L-triiodothyronine in the peripheral tissues.

2. Thyrotropic Hormone

This substance sustains the activity of the thyroid gland, promoting increased uptake of inorganic iodine and release of organically bound iodine. Two groups of workers, Condliffe and Bates (35) and Pierce et al. (36) have succeeded in the long elusive task of purifying bovine TSH to a high degree. Even this highly purified preparation may be developed by starch gel electrophoresis into two or more active bands (36), suggesting that thyrotrophic effects may reside in a family of closely related molecules rather than being confined to a single fixed configuration of amino acids. Purified thyrotropic fractions have not as yet been characterized chemically, except that a molecular weight between 26,000 and 30,000 is indicated by electro dialysis studies in starch gel (37).

Although no specific therapeutic uses for thyrotropin have been established it has found diagnostic application in the differentiation of hypothyroidism due to pituitary failure and that due to primary thyroid failure.

A discussion of the role of TSH in thyroid function is given under "Thyroid Pituitary Relationships."

C. The Effects of Thyroid Hormones on the Respiration of Tissue

Saljous (38) as early as 1907 discussed the importance of tissue responses to endocrine function. Tissue respiration studies have been carried out: - 1. when the injection of the thyroid drug was given to the animal in vivo and, 2. when the slices of tissue were incubated with a thyroid drug in vitro. A review of these methods follows.

1. In Vitro Studies

Reports on the response of tissues to in vitro addition of thyroid drugs are conflicting. The majority have found no increase of oxygen uptake by excised tissue when thyroxine was added (39-45). However, a few other workers have observed an increase (46-47). Thyroglobulin, on the other hand, has generally been observed to increase QO_2 of tissue slices (40-42), although Williams-Ashman (48) reported no such response.

Variations in the conditions of investigation influence the results. Canzanelli, Guild and Rapport (41) found that thyroxine in serum caused a stimulatory effect. This was also observed by Williams and Whittenberg (42) in about one-half of their experiments. Yoshihiro (49), working with dog adrenals, found an increased respiration with low concentrations of thyroxine and a decrease with high concentrations. Weinstein and Lein (50) found that thyroxine caused no effect on the QO_2 of diaphragm for the first two hours of incubation but thereafter a stimulation occurred. Barker (51) observed that the oxygen consumption of kidney slices incubated with thyroxine was higher than untreated controls. He incubated the tissues with thyroxine for varying periods of time at 5° C and then raised the

temperature to 37° C for determining the oxygen consumption. He also observed that if DL-alanine was added to the medium, control slices were maintained at the initial level of respiration. There have also been contradictory reports regarding the effects of triiodothyroacetic acid (triac) and tetraiodothyroacetic acid (tetrac). Thibault and Pitt-Rivers (52) reported that tetrac and triac raised the oxygen consumption of kidney slices without any latent period. Barker and Lewis (53) on the other hand reported no immediate effect on oxygen consumption of liver, kidney, and heart when these compounds were added.

From the above review of the in vitro work it is evident that the results are varied and often contradictory. In view of this Barker (54) concluded that "the very diversity of experimental procedures indicates that no readily reproducible observations have been made."

2. In Vivo Studies

The first studies on oxygen consumption of tissues from animals which were administered thyroid hormone in vivo dates back more than 30 years. Rohrer (55) in 1924 reported in the literature that he found an acceleration of oxygen uptake of liver, kidney and muscle isolated from mice treated with desiccated thyroid. A survey of the effects of thyroid hormone on the three tissues studied in this investigation follows.

a. Diaphragm

Foster (56) in 1927 found that bits of diaphragm from thyroidectomized rats had a lower QO_2 than diaphragm from normal rats. This is a

general finding by all later workers. The feeding of desiccated thyroid to rats increases diaphragm QO_2 (58) (59). McEachern (57) found that injection of thyroxine increased the QO_2 of diaphragm 75%. A number of other workers have also found that injection of thyroxine increased the respiration of diaphragm (58, 60-65). Triiodothyronine has a similar effect (63) (64).

There has been general agreement, therefore, that the oxygen consumption of the diaphragm is significantly lowered in hypothyroid states whereas administration of a thyroid hormone either by the oral route or by injection causes an increase in oxygen consumption of the diaphragm.

b. Heart

The respiration of heart slices has received considerable attention due possibly to the well-known effect of thyroid hormones on heart function. McEachern and Andrus (66) in 1931 compared the oxygen consumption of isolated beating auricles from normal guinea pigs with that from thyroxine treated animals. The QO_2 for the latter group was about 20% higher. Gerard and McIntyre (67) obtained similar results with dogs. Barker and Klitgaard (61) showed that the left ventricle QO_2 was significantly reduced by thyroidectomy and that treatment of thyroidectomized rats with thyroxine caused a maximum oxygen consumption in the heart. Ullrick and Whitehorn (59) observed no difference between the QO_2 of the atrium and ventricle of normal rats. However atrial oxygen consumption increased 77% when the rats were fed dried thyroid. Goh and Dallam (47) found that the oxygen consumption of the left ventricle was 25% greater than that of the right ventricle and atrium in normal animals. Hypothyroid hearts showed a

decrease in oxygen consumption of the atrium and both ventricles. In hyperthyroid animals the oxygen consumption of the whole heart increased but not proportionately for each area of the heart. Barker (63) found that triiodothyronine had the same qualitative effect on heart QO_2 as did thyroxine but that only one quarter the dose of triiodothyronine was needed to give the same effect as thyroxine. Whaley, Hart and Klitgaard (64) compared the time response curve for heart when the animal was treated with a single dose of triiodothyronine and when a single dose of thyroxine was given. Both drugs caused essentially the same effects except that triiodothyronine acted more quickly and for a shorter time. Barker (51) found that heart responded more dramatically and earlier than other tissues when a single dose of triiodothyronine, tetrac, or triac was administered. Saunders et al. (68) were not able to show a significant difference between the QO_2 of heart from normal and thyroidectomized rats. They obtained a stimulant effect with triiodothyronine and triac but did not find that heart responded more than liver as was observed by Barker and Lewis (53). Barker and Lewis (53) found that tetrac and triac injected into thyroidectomized rats over a period of several days had the same order of activity as an equimolar dose of thyroxine. They also observed that triac and tetrac did not appear to produce an immediate metabolism-stimulating effect on the heart. Dunne and Tapely (65) injected thyroidectomized rats with D- and L-thyroxine. They observed that the L isomer gave a higher QO_2 than the D isomer.

In the above work it is generally agreed that the administration of thyroid hormones to thyroidectomized rats increases the QO_2 of the heart. Thyroidectomy decreased the QO_2 of the heart in all reports except that of Saunders et al. (68). Saunders et al. (68) and Barker and Lewis (53)

disagree as to the difference of response of liver and heart. There are also differences between the findings of Ullrick and Whitehorn (59) and Goh and Dallam (47) regarding the oxygen consumption of different areas of the heart.

c. Brain

There is considerable disagreement in the literature regarding the effects of altered thyroid states on brain QO_2 . Most of the work carried out supports the claim that alteration in the thyroid state does not influence the QO_2 of the brain. Spirtes (69) fed female guinea pigs dried thyroid and obtained no increase in the QO_2 of the brain slices. Gordon and Heming (58) observed no change in the QO_2 of the brain of normal animals after oral thyroid or injected thyroxine. Brophy and McEachern (70) made similar findings. Fazekas, Graves, and Ahlman (70a) fed propylthiouracil to rats during pregnancy and lactation and then observed the effects on the young. This treatment caused no significant difference in the QO_2 of the brain. These workers also observed no difference in the QO_2 of brain after injections of thyroxine. Barker and Klitgaard (61) and Barker and Schwartz (62) found that neither thyroidectomy nor injection of thyroxine produced any significant differences in the QO_2 of the brain. Barker (63) also obtained negative results with triiodothyronine. Hoexter (71) agreed with others already cited that thyroidectomy caused no effect on brain QO_2 .

Some of the variations noted above may be due to differences in handling the tissues. Gordon and Heming (58) and Hoexter (71) do not mention how the brain tissue was prepared. Spirtes (69) and Brophy and McEachern (70) used slices whereas all the rest used minces. It may also be of significance that Barker and Klitgaard (61), Barker and Schwartz (62) and Barker

(63) used ether and exsanguination to sacrifice their animals.

Positive results for response of brain to altered thyroid states have been observed only by two groups of workers. Cohen and Gerrard (72) observed that oxygen consumption of brain increased after rats were fed thyroid. Rossiter (73) prepared brain brei by mincing the brain on a hot plate at 38° C with a bone spatula or prepared brain dispersions by mixing the brain at 4° C with Ringer-Phosphate and then pressed it through muslin. In the presence of glucose, sodium pyruvate and sodium succinate, brain brei from thyroid- and vitamin B₁-treated rats had a higher O₂ uptake than brei from controls that received only vitamin B₁. If no vitamin B₁ was given, the increase was smaller with glucose and disappeared with pyruvate. With dispersion preparations from similarly treated animals he observed no increase in O₂ uptake.

It can be seen, then, that the number of workers reporting negative results with brain is much greater than those who claim positive results.

In addition to the tissues discussed here much work has been carried out on other tissues. Details regarding these investigations may be found in the excellent review by Barker (54) who in 1951 made a survey of this work up to that time.

2. Studies on Adrenal Thyroid Interrelationships

For some time it has been recognized that there is an interrelationship existing between adrenal and thyroid function. Hoffmann, Hoffmann, and Talesnik (74) found that adrenalectomy decreased oxygen consumption 10% as measured by BMR studies. The BMR was decreased 20% when rats were subjected to both adrenalectomy and thyroidectomy. Adrenal

cortical hormones raised the metabolic rate of thyroidectomized-adrenalectomized rats. Adrenalectomy performed at the time when thyroxine produced its greatest acceleration of respiration lead to a decrease of oxygen consumption in spite of continuous thyroxine administration. Ganju and Lockett (75) observed that when normal and thyroidectomized mice were fed dried thyroid the oxygen uptake of both was increased. However the oxygen uptake of adrenalectomized mice was not increased with this treatment. Administration of adrenal cortical hormones to thyrotoxic rats by Doisy and Lardy (76) caused no change in the BMR of the rats investigated.

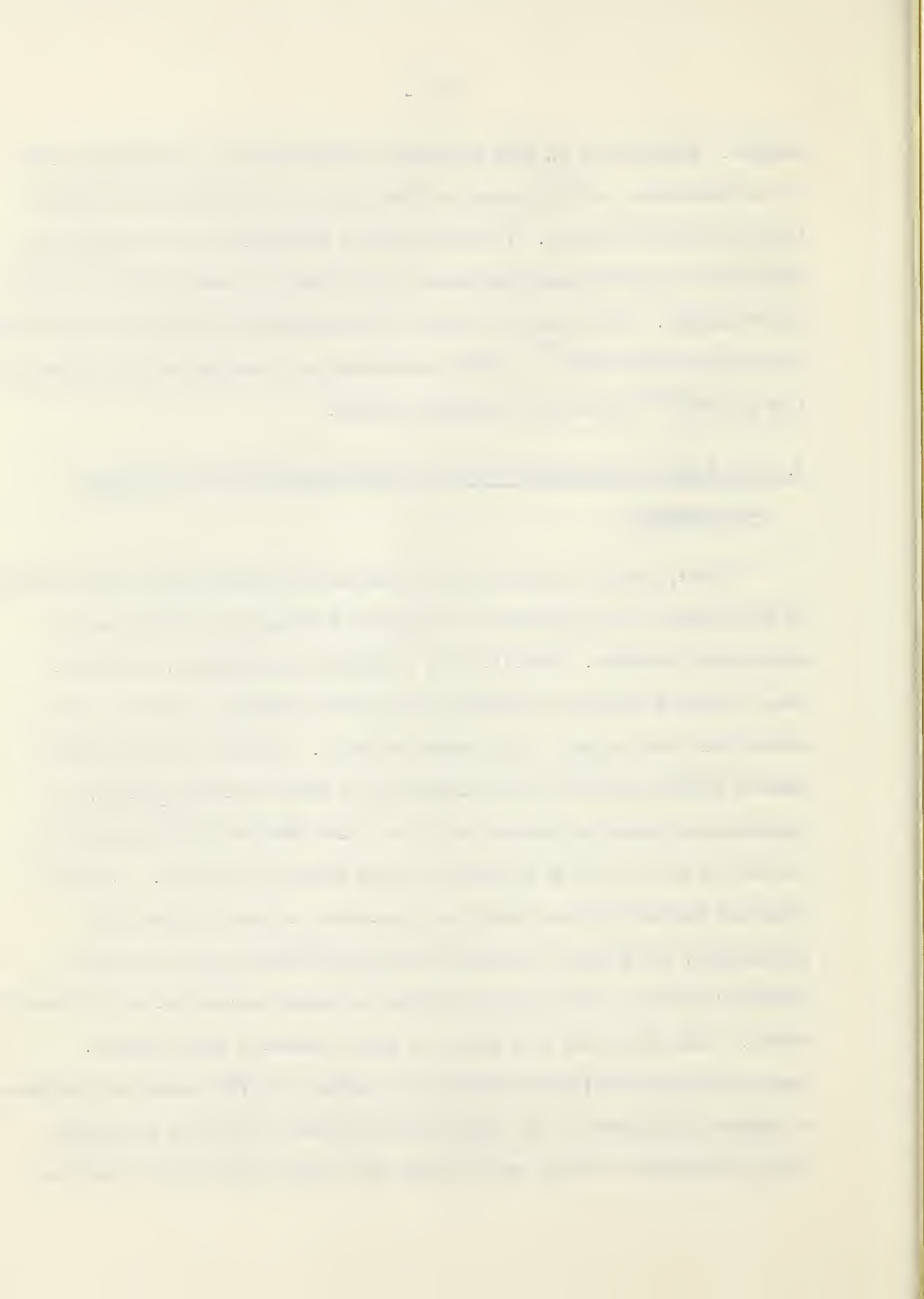
Kowalewski and Bekesi (77) investigated the effect of cortisone at the tissue level in rats. They found that cortisone significantly depressed the oxygen uptake of diaphragm, liver, myocardium, and kidney slices. Lacroix and Leusen (78) found that the QO_2 of the heart was markedly depressed while that of the diaphragm was increased when male rats were given cortisone. Cortisone treatment of thyroidectomized rats depressed the myocardial respiration further but the oxygen uptake of the diaphragm was stimulated under this treatment. When the same cortisone treatment was given to thyroidectomized male rats which received thyroxine regularly so that a normal oxygen uptake of myocardium and diaphragm existed, identical observations were made as in normal animals i.e. myocardium showed the same decrease in oxygen consumption and the QO_2 of the diaphragm was clearly stimulated.

Some mention should be made of the effects of adrenaline on thyroid activity. Swanson (79) found that thyroidectomy inhibited and thyroxine potentiated the calorogenic effect of adrenaline as determined by BMR

studies. Eskelson et al. (80) found that administration of adrenaline alone or in combination with thyroxine or TSH produced a lowering of the blood level of thyroid hormone. This response to adrenaline was interpreted as being due to the increased utilization of circulating thyroid hormone caused by adrenaline. Ackerman and Arons (81) catheterized the thyroid veins of dogs and measured the PBI^{131} . After administration of adrenaline and noradrenaline the PBI^{131} levels were markedly raised.

3. The Effect of Hypophysectomy and TSH Administration on Oxygen Consumption

Eitel, Krebs, and Loeser (82) showed that thyroid tissue slices undergo hyperplasia when incubated in serum for 24 hours in the presence of thyrotropic hormone. Paal (83) (84) in similar experiments, found that when thyroid tissue was incubated with anterior pituitary extract a three to four fold rise in QO_2 of the tissue occurred. He also found that while neither TSH nor thyroid tissue alone had any effect on liver in vitro, the incubation of guinea pig thyroid and liver, plus TSH for 24 hours caused the QO_2 of the liver to be as much as twice that of the normal. Paal also observed that after 3 days injection of anterior pituitary extract into guinea pigs, the oxygen consumption of thyroid tissue taken from such animals was three to four times as great as that of normal guinea pig thyroid tissue. This effect was also seen to a lesser extent in other tissues. Anderson and Alt (85) also found that incubation with TSH caused an increase in oxygen consumption in the thyroid tissue but not in the liver and kidney. Victor and Anderson (86), using female rats, noticed that both kidney and



liver tissue showed a decrease in oxygen consumption after hypophysectomy. Canzanelli and Rapport (87) concurred in the findings already cited in that they found that in vivo injection of TSH caused thyroid QO_2 as well as liver QO_2 to be elevated. Again in vitro administration of TSH increased thyroid QO_2 but not the QO_2 of the liver. Macleod and Reiss (88) found that hypophysectomy did not lower brain oxygen consumption as it did liver oxygen consumption. After treatment of hypophysectomized rats with TSH, the oxygen consumption of brain was found to be increased, as compared to normal unoperated animals, between 5-8 days after commencement of treatment. The liver oxygen uptake was restored or slightly increased above the normal. Jandorf and Williams (89) gave rats thiouracil, TSH, or both. The oxygen consumption of the thyroid was raised more when both drugs were administered. The oxygen uptake of liver and diaphragm was increased with TSH but the effect was abolished when thiouracil was administered with the TSH. Thiouracil alone gave no effect. Von Bertalanffy and Estwick (90) observed that the oxygen consumption of skeletal and cardiac muscle was reduced markedly after hypophysectomy. The recent findings of Hart et al. (91) agree with the earlier workers in that they observed an increase in the oxygen consumption of thyroid tissue when TSH was added to it in vitro.

In summary, it may be said that hypophysectomy reduces the QO_2 of liver, kidney, heart, and skeletal muscle. It also has been observed by all workers that thyroid QO_2 is increased when TSH is administered in vivo or in vitro. Other tissues respond only when TSH is administered in vivo and not when the drug is added to the Warburg vessel.

III STATEMENT OF THE PROBLEM

A close relationship exists between thyroid activity and the rate of metabolism of the organs of the body. Tissue respiration studies lend themselves well to investigations of the mechanism of thyroid activity. Although there has been extensive work using desiccated thyroid and thyroxine and a small amount using triiodothyronine, the reported results are variable and frequently contradictory. The basic biochemistry of thyroid activity is still so obscure that Goldman and Gilman (92) comment:- "The fundamental mechanism of action of the thyroid hormone is completely unknown." It was hoped that the application of a modified Warburg technique would provide data that would help to clarify the uncertainties of thyroid action.

Standard Warburg methods for the in vitro study of tissue respiration involve suspension of the tissue in a fluid medium. This medium influences the reaction of the tissue. Huston and Martin (93) have proposed a technique whereby the tissues to be studied are excised, spread on fibre glass mats, and placed in an atmosphere of oxygen in a special type of Warburg flask. This procedure has been used successfully in pharmacological studies (94-97). Since it is felt that the results obtained using this technique more closely approximate the in vivo situation, it was decided to investigate the effects of altered thyroid states on tissue respiration by this more sensitive method.

IV EXPERIMENTAL

1. Method of Handling the Animals and Drugs

Young male albino rats of the Wistar strain weighing 150-200 grams at the time of thyroidectomy were used. Thyroidectomy was performed under Nembutal anesthesia (40 mg. /Kg.) for the first three series and under ether anesthesia in the last three series. The rats were given 1% CaCl_2 in their drinking water for two weeks following the surgical procedure. Complete development of the athyroid state was insured by waiting for at least four weeks after surgery before the experiments were begun. Each of the series from I-IV were divided into four groups. These groups consisted of normal animals, normal animals treated with L-triiodothyronine, thyroidectomized animals, and thyroidectomized animals treated with L-triiodothyronine.

Adrenalectomy was performed by the dorsal route while the animals were under ether anesthesia.

Hypophysectomized rats of the Sprague-Dawley strain were obtained from Hormone Assay Laboratories, Chicago, Ill. and E.G. Steinhilber and Co., Inc., Oshkosh, Wisconsin.

The series into which the rats were divided together with procedures pertinent to each series are listed below.

Series I

No. of Animals	- 40.
Diet	- Purina Chow ad libitum.
Drug Administration	- L-triiodothyronine (5.13 mg./Kg.) subcutaneously 4 days before sacrificing.
Tissues Investigated	- Diaphragm and heart.
Duration	- 10 weeks.

Series II

No. of Animals	- 42.
Diet	- Purina Chow ad libitum.
Drug Administration	- L-triiodothyronine (5.13 mg./Kg.) subcutaneously 7 days before sacrificing.
Tissues Investigated	- Diaphragm and heart.
Duration	- 5 weeks.

Series III

No. of Animals	- 40.
Diet	- Wheat 65.5%
	Soybean Oil Meal 32.0%
	Brewers Yeast 1.0%
	Ca ₃ (PO ₄) ₂ 1.0%
	NaCl 0.5%
	Vitamin A 200 i. u. per kilo

Drug Administration	- L-triiodothyronine (5.13 mg./Kg.) subcutaneously 4 days before sacrificing.
Tissues Investigated	- Diaphragm and heart.
Duration	- 10 weeks.

Series IV

No. of Animals	- 40.
Diet	- Purina Chow ad libitum.
Drug Administration	- L-triiodothyronine (5.13 mg./Kg.) subcutaneously 4 days before sacrificing.
Tissue Investigated	- Brain.
Duration	- 9 weeks.

Series V

No. of Animals	- 44.
Groups	- Normal, adrenalectomized, thyroidectomized, and thyroidectomized-adrenalectomized.
Diet	- Purina Chow ad libitum.
Drug Administration	- None.
Tissues Investigated	- Diaphragm, heart and brain.
Duration	- 3 weeks.

Series VI

No. of Animals	- 13.
Groups	- Normal and hypophysectomized.
Diet	- Oranges, bread, carrots, milk, oatmeal, and Purina Chow ad libitum.

Drug Administration - None.
Tissues Investigated - Diaphragm, heart and brain.
Duration - 8-16 days after hypophysectomy.

Series VII

No. of Animals - 21.
Groups - Normal, normal-treated, hypophysectomized, and hypophysectomized-treated.
Diet - Oranges, bread, carrots, milk, oatmeal, and Purina Chow ad libitum.
Drug Administration - Thyrotropin (1 USP unit per rat) subcutaneously 4 and 24 hours before sacrificing.
Tissues Investigated - Diaphragm, heart and brain.
Duration - 9-17 days after hypophysectomy.

2. Tissue Respiration Apparatus

In order to measure the respiration of tissues in contact with oxygen, it is necessary to have the samples suspended in such a manner as to assure maximal contact of the tissue with the gas. The slices of tissue were spread out evenly on fibre glass mats and placed in wide mouthed flasks similar to those designed by Huston and Martin (93) but modified by Huston (98). The vessel, of about 19 ml. capacity, was attached to a standard Warburg manometer by a glass adapter. A removable tray rested on the bottom of the vessel. The mat bearing the tissue was placed on the removable tray.

3. Method of Tissue Respiration

The animals were weighed, and then killed by decapitation. The diaphragm, heart, and brain were quickly excised and transferred to a moist cold chamber (2-5° C). The diaphragm was cleaned of connective tissue and adherent fat and pieces were cut parallel to the fibres with scissors. Heart and brain were cut through a template with a razor blade. The tissues were spread out carefully on fibre glass mats, weighed on a Gram-atic balance and kept in moist Petri dishes in the cold chamber until all had been weighed. The mats were then placed on top of the removable tray in the Huston-Martin flasks which had been previously warmed to 37° C. on a hot plate. The oxygen consumption was determined by the direct method of Warburg using a gas phase of oxygen. The determinations were carried out at a temperature of 37.9° C. Carbon dioxide was absorbed by 0.2 ml. of 10% KOH which was absorbed on a piece of filter paper on the bottom of the flask. The removable tray contained Krebs Ringer Phosphate (99). The operations and weighings required approximately 20 minutes. The equilibrium period in the water bath was approximately 15 minutes, during which time it proved essential that all ground glass connections be thoroughly tightened. Readings were taken at 10 minute intervals for 80 minutes.

4. Calculation of Results

Warburg manometers record changes in pressure of gas in the flask. The flask constant, coupled with the weight of the tissue is used

THE HISTORY OF THE

REIGN OF KING CHARLES THE FIRST

IN THE YEAR 1649

BY JOHN BURNET

OF THE UNIVERSITY OF OXFORD

IN TWO VOLUMES

THE SECOND VOLUME

CONTAINING THE

REIGN OF KING CHARLES THE FIRST

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THE SECOND VOLUME

to determine a standardized QO_2 . The QO_2 in this investigation expresses ml. of oxygen consumed per gram of tissue (wet weight) per hour. Since the rate of respiration in artificial media declines with time, the QO_2 value for zero time must be obtained by straight line extrapolation of the rates during the experimental period. The rate of respiration has been depressed by the cold during the operation procedure and returns to a maximum at the conclusion of thermal equilibrium. This rate of respiration is therefore the closest approximation to that in situ.

Mean QO_2 values of each tissue investigated were determined. Any difference from normal values noted was tested for significance by the normal approximation or in the case of the last series by the Student's "t" test (100). The probability of 0.05 was selected as the point of significance.

V. RESULTS

Nielson et al. (101) found that diaphragm, heart and brain had reached their basal level of respiration before 28 days had elapsed after thyroidectomy. To insure that the basal level of respiration had been obtained, all rats in our investigation were left at least 28 days after thyroidectomy before experiments were begun.

1. The Effect of L-triiodothyronine on Tissue Respiration of Diaphragm and Heart

In the first series (Table I) L-triiodothyronine was injected 4 days before sacrificing. Treatment with the drug increased the respiration of the diaphragm significantly in both the normal and thyroidectomized animals. Treatment, however, made no significant difference to the respiration of the heart. Thyroidectomy did not lower the respiration of the heart or the diaphragm significantly below that of normal.

In the second series all conditions were the same as in the first series with the exception that the L-triiodothyronine was administered 7 days before the animals were sacrificed. It can be seen from Table I that a significant decrease in the respiration of the diaphragm and heart occurred after thyroidectomy. The respiration of the diaphragm and heart from thyroidectomized animals receiving L-triiodothyronine was significantly increased above that of thyroidectomized animals. However, L-triiodothyronine had no significant effect on the respiration of the diaphragm and

heart of normal animals.

2. Effects of a Special Diet on Tissue Respiration of Diaphragm and Heart

Leblond and Eartly (102) reported that if thyroidectomized rats were fed a diet low in iodine and deficient in animal protein, their body weight was reduced as compared to those thyroidectomized rats which received Purina Fox Chow. These rats also showed symptoms which were quite comparable to the classical descriptions of athyroidism. These workers suggested that there were extrathyroidal areas in the rat which converted iodine and tyrosine into the thyroid hormones. They also suggested that animal protein would contain appreciable amounts of thyroxine.

Because of these reports of Leblond and Eartly it was felt that a study of the effects on tissue respiration of a diet low in iodine and deficient in animal protein was desirable. The ingredients of the diet have been mentioned earlier. The results of Series III are contained in Table I. Thyroidectomy decreased the respiration of the diaphragm 32% below that of normal in Series II and only 16% below that of normal in the animals on the special diet (Series III). Thyroidectomy depressed the heart respiration 7% in Series II and 10% in the animals maintained on special feed (See Table V). It appears then that the special diet did not affect the respiration of diaphragm and heart appreciably.

When the other aspects of Series III are considered, it can be seen that results similar to those of Series II were obtained. L-triiodothyronine was administered 4 days before sacrificing. Thyroidectomy caused a significant decrease in the respiration of both the heart and diaphragm.

TABLE I

EFFECT OF L-TRIODOTHYRONINE ON THE RESPIRATION OF DIAPHRAGM AND HEART

Figures represent mean QO_2 values in ml O_2 per gram wet weight per hour.

L-triiodothyronine injected prior to sacrifice, 4 days in Series I, 7 days in Series II and 4 days in Series III.

TX									
- thyroidectomized									
T3									
S									
- Significantly different ($p \leq 0.05$)									
Tissue	No. of Experi-ments	Series I	Sig.	No. of Experi-ments	Series II	Sig.	No. of Experi-ments	Series III	Sig.
Diaphragm									
Normal	39	1.45 \pm 0.35	}s	27	1.72 \pm 0.31	}s	29	1.67 \pm 0.48	}s
Normal + T3	38	1.71 \pm 0.42		23	1.86 \pm 0.34		26	2.08 \pm 0.30	
TX	32	1.32 \pm 0.30	}s	28	1.16 \pm 0.31	}s	27	1.40 \pm 0.23	}s
TX + T3	25	1.70 \pm 0.41		27	1.91 \pm 0.51		20	1.90 \pm 0.29	
Heart									
Normal	42	2.42 \pm 0.36	}s	30	2.67 \pm 0.26	}s	30	2.75 \pm 0.25	}s
Normal + T3	36	2.54 \pm 0.32		30	2.79 \pm 0.34		32	2.78 \pm 0.26	
TX	32	2.43 \pm 0.27	}s	32	2.47 \pm 0.25	}s	27	2.45 \pm 0.25	}s
TX + T3	33	2.50 \pm 0.27		26	2.73 \pm 0.41		24	2.60 \pm 0.32	

The drug increased the respiration of the diaphragm significantly in both normal and thyroidectomized animals. The respiration of the heart was not significantly increased when normal and thyroidectomized animals were treated with the drug. It should be noted, however, that the difference between the respiration of the heart from the thyroidectomized group and the respiration of the heart from the thyroidectomized-treated group is significant at the 93% level.

3. Effect of L-triiodothyronine on Brain Respiration

As has been pointed out in the literature survey the majority of workers have obtained no significant decrease in the respiration of the brain slices of thyroidectomized rats as compared to normal rats when investigated by standard Warburg procedures. However using the Huston-Martin technique a significant decrease was shown in the respiration of brain slices from thyroidectomized rats as compared to normal brain slices. Triiodothyronine increased the respiration of the brain significantly in both the normal and thyroidectomized groups.

4. Effect of Thyroidectomy, Adrenalectomy, and Combined Thyroidectomy and Adrenalectomy on Tissue Respiration

The summary of the results from this study are contained in Table III.

a. Diaphragm

The respiration of the diaphragm from thyroidectomized, adrenalectomized, and thyroidectomized-adrenalectomized rats was significantly

TABLE II

EFFECT OF L-TRIODOETHYRONINE

ON THE RESPIRATION OF BRAIN SLICES

Figures represent mean QO_2 values in ml O_2 per gram wet weight per hour.

L-triiodothyronine injected 4 days prior to sacrifice.

TX - thyroidectomized
 T_3 - L-triiodothyronine
 S - significantly different ($p \leq 0.05$)

Tissue	No. of Experiments	Series IV	Sig.
Brain			
Normal	41	2.81 ± 0.26	<div style="display: inline-block; vertical-align: middle;"> $\left. \begin{array}{l} \}^{\text{S}} \\ \}^{\text{S}} \end{array} \right\}^{\text{S}}$ </div>
Normal + T_3	35	2.93 ± 0.26	
TX	39	2.67 ± 0.22	
TX + T_3	37	2.82 ± 0.29	

EFFECT OF ADRENALECTOMY, THYROIDECTOMY, AND
COMBINED THYROIDECTOMY AND ADRENALECTOMY ON
THE RESPIRATION OF DIAPHRAGM, HEART, AND BRAIN

ADX - adrenalectomized
TX - thyroidectomized
S - significantly different ($p \leq 0.05$)

- 32 -

depressed below the respiration of normal diaphragm in all cases. The QO_2 of diaphragm from thyroidectomized-adrenalectomized rats was significantly lower than the respiration of diaphragm slices from adrenalectomized rats. No significant difference occurred between the diaphragm respiration from thyroidectomized rats and that of thyroidectomized-adrenalectomized rats.

b. Heart

The respiration of the heart from thyroidectomized, adrenalectomized, and thyroidectomized-adrenalectomized rats was significantly depressed below the respiration of normal heart in all cases. The QO_2 of heart from thyroidectomized-adrenalectomized rats was significantly lower than the respiration of heart from adrenalectomized rats. No significant difference occurred between the heart respiration from thyroidectomized rats and that of thyroidectomized-adrenalectomized rats.

c. Brain

The respiration of the brain from thyroidectomized and thyroidectomized-adrenalectomized rats was significantly depressed below the respiration of brain from normal animals. Adrenalectomy did not significantly depress the respiration below that of normal ($p > 0.16$). The respiration of brain from thyroidectomized-adrenalectomized rats was significantly lower than the respiration of brain slices from adrenalectomized rats. No significant difference occurred between adrenalectomized and thyroidectomized-adrenalectomized rats.

5. Effects of Hypophysectomy and Thyrotropin on Tissue Respiration

In order to determine whether hypophysectomy affects tissue respiration a preliminary group of rats was investigated. Six normal and seven hypophysectomized rats were used and the results of these experiments are found in Series VI, Table IV. Hypophysectomy significantly depressed the respiration of heart and brain tissue below that of normal. Diaphragm QO_2 was not significantly depressed by hypophysectomy but the difference was approaching significance ($p\ 0.09$).

The work in Series VII was undertaken to determine the effects of thyrotropin (TSH) administration on the tissue respiration of normal and hypophysectomized rats. Normal and hypophysectomized animals were treated with TSH four hours before sacrificing and another two groups of normal and hypophysectomized animals were treated with TSH twenty-four hours before sacrificing. Only trends have been established in the majority of cases. Diaphragm was the only tissue whose respiration was significantly lowered below that of normal by hypophysectomy. Hypophysectomy depressed the respiration of heart slices below that of normal but the difference in respiration is only significant at the 94% level. The QO_2 of brain was depressed by hypophysectomy but not significantly. Administration of TSH to normal rats four hours before sacrificing caused a significant increase in the respiration of the brain. The increase in the case of diaphragm approached significance ($p\ 0.12$) while the QO_2 of the heart was increased but not significantly. Administration of TSH to normal rats twenty-four hours before sacrificing caused no significant change in the respiration of the diaphragm, heart and brain.

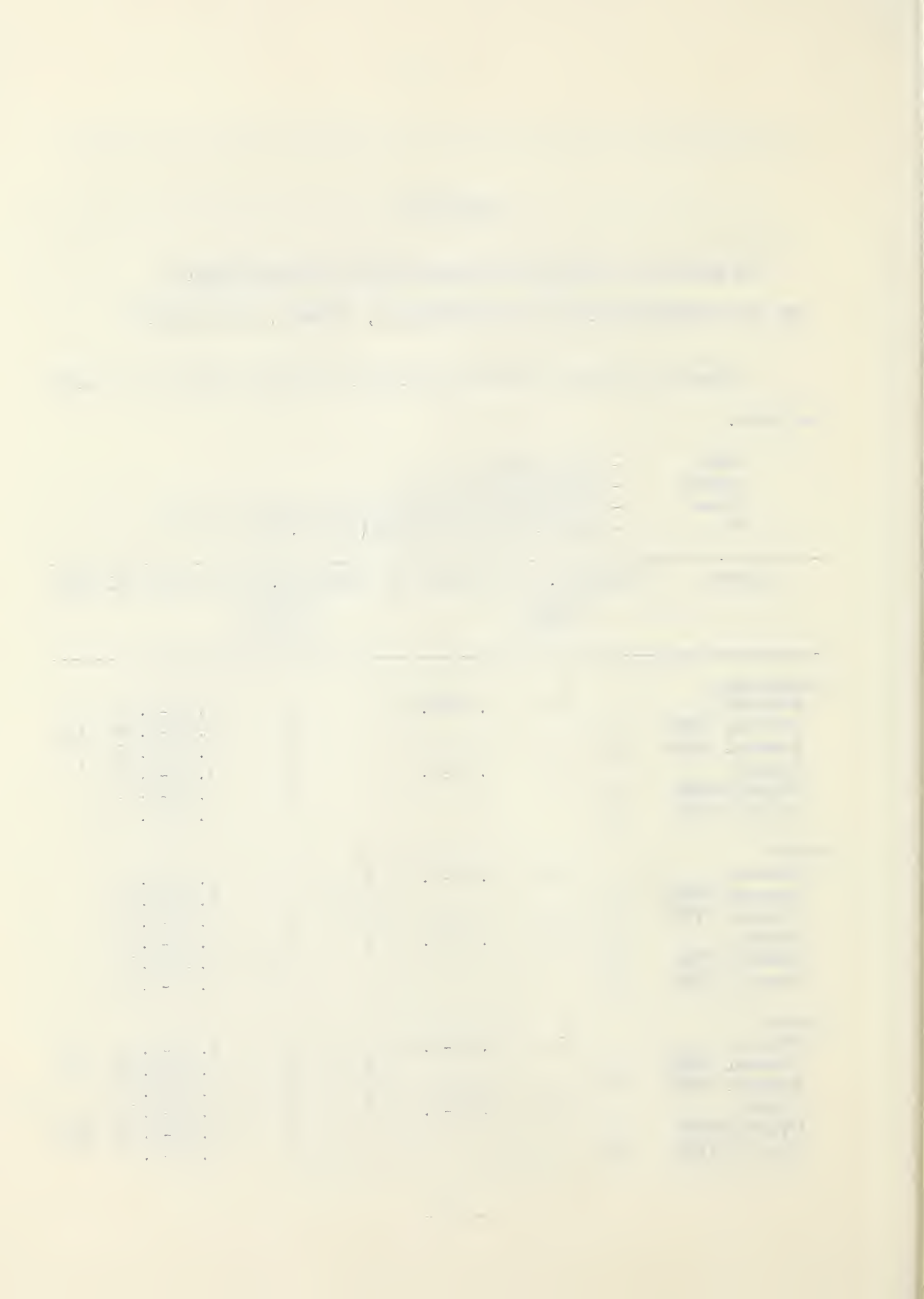
TABLE IV

EFFECT OF HYPOPHYSECTOMY AND THYROTROPIN
ON THE RESPIRATION OF DIAPHRAGM, HEART, AND BRAIN

Figures represent mean QO_2 values in ml O_2 per gram wet weight per hour.

TSH - thyrotropin
HypoX - hypophysectomized
Time - hours between injection time and sacrifice
S - significantly different ($p \leq 0.05$)

Tissue	Time	No. of Experi- ments	Series VI	Sig.	No. of Experi- ments	Series VII	Sig.
Diaphragm							
Normal		10	1.79 ± 0.24		8	2.07 ± 0.28	} S
Normal + TSH	4				8	2.30 ± 0.24	
Normal + TSH	24				6	2.08 ± 0.22	
HypoX		13	1.57 ± 0.30		6	1.63 ± 0.31	
HypoX + TSH	4				4	1.63 ± 0.23	
HypoX + TSH	24				7	1.65 ± 0.24	
Heart							
Normal		12	2.51 ± 0.17	} S	8	2.89 ± 0.21	} S
Normal + TSH	4				8	2.99 ± 0.22	
Normal + TSH	24				7	2.78 ± 0.13	
HypoX		14	2.19 ± 0.16		6	2.60 ± 0.27	
HypoX + TSH	4				4	2.41 ± 0.21	
HypoX + TSH	24				7	2.49 ± 0.34	
Brain							
Normal		12	2.80 ± 0.26	} S	8	2.87 ± 0.21	} S
Normal + TSH	4				8	3.20 ± 0.29	
Normal + TSH	24				6	3.08 ± 0.27	
HypoX		14	2.53 ± 0.31		6	2.73 ± 0.24	} S
HypoX + TSH	4				4	2.79 ± 0.19	
HypoX + TSH	24				7	2.44 ± 0.09	



When TSH was administered to hypophysectomized rats four hours before sacrificing no significant change was observed in the QO_2 of the diaphragm, heart or brain. Only two animals were injected however. Administration of TSH to hypophysectomized rats twenty-four hours before sacrificing produced no significant change in the QO_2 of the diaphragm. However, a significant depression occurred in the QO_2 of the brain. A slight increase in the QO_2 of the heart occurred when TSH was given twenty-four hours before sacrificing.

6. Comparison of the Results When the Mean QO_2 Values are Expressed as a Percentage of Their Controls

In Tables V, VI, and VII, and Figures I and II, a summary is presented of the results expressed as a percentage of the control mean QO_2 .

Examination of Table V and Figure I discloses that treatment of thyroidectomized rats with L-triiodothyronine returned the respiration of the diaphragm to above that of the normal diaphragm and, indeed, the respiration approached that of the diaphragm from normal rats treated with L-triiodothyronine. Treatment of thyroidectomized rats with L-triiodothyronine returned the respiration of heart to slightly above normal in the first two series. The respiration approximated normal in the third series (96%). Treatment of thyroidectomized rats with L-triiodothyronine returned the respiration of the brain to normal. The decreased respiration of all tissues from thyroidectomized rats, then, is increased to the normal level of respiration by treatment with L-triiodothyronine.

Adrenalectomy, thyroidectomy, and a combination of thyroidectomy and adrenalectomy decreased the respiration of all three tissues. (Table VI and Figure II). The difference in sensitivity of the three tissues to

TABLE V

SUMMARY OF MEAN QO_2 VALUES EXPRESSED AS
A PERCENTAGE OF CONTROLS

N - normal
TX - thyroidectomized
 T_3 - L-triiodothyronine
S - significantly different ($p \leq 0.05$)

Tissue	% of	Series I	Series II	Series III
Diaphragm				
N + T_3	N	118 S	108	125 S
TX	N	91	67 S	84 S
TX + T_3	N	117	111	114
TX + T_3	TX	129 S	165 S	136 S
Heart				
N + T_3	N	105	105	101
TX	N	100	92 S	89 S
TX + T_3	N	103	102	96
TX + T_3	TX	103	111 S	106
Brain				
		Series IV		
N + T_3	N	104 S		
TX	N	95 S		
TX + T_3	N	100		
TX + T_3	TX	106 S		

TABLE VI

SERIES V

SUMMARY OF MEAN QO_2 VALUES EXPRESSED AS
A PERCENTAGE OF NORMAL

TX - Thyroidectomized
ADX - Adrenalectomized
S - Significant difference ($p \leq 0.05$)

Group	Diaphragm	Heart	Brain
ADX	89 S	90 S	95
TX	73 S	83 S	92 S
TX - ADX	68 S	81 S	87 S

TABLE VII

SUMMARY OF MEAN QO_2 VALUES EXPRESSED AS
A PERCENTAGE OF CONTROLS

N - Normal
H - Hypophysectomized
TSH - Thyrotropin
S - Significant difference ($p \leq 0.05$)
Time - Hours between injection time and sacrifice

	Group	Time	% of	Diaphragm	Heart	Brain
Series VI	H		N	88	87 S	90 S
Series VII	N + TSH	(4 hrs)	N	111	103	112 S
	H		N	79 S	90	95
	H + TSH	(4 hrs)	N	79	83	97
	H + TSH	(4 hrs)	H	100	93	102
	N + TSH	(24 hrs)	N	100	96	107
	H + TSH	(24 hrs)	N	80	86	85
	H + TSH	(24 hrs)	H	101	96	89



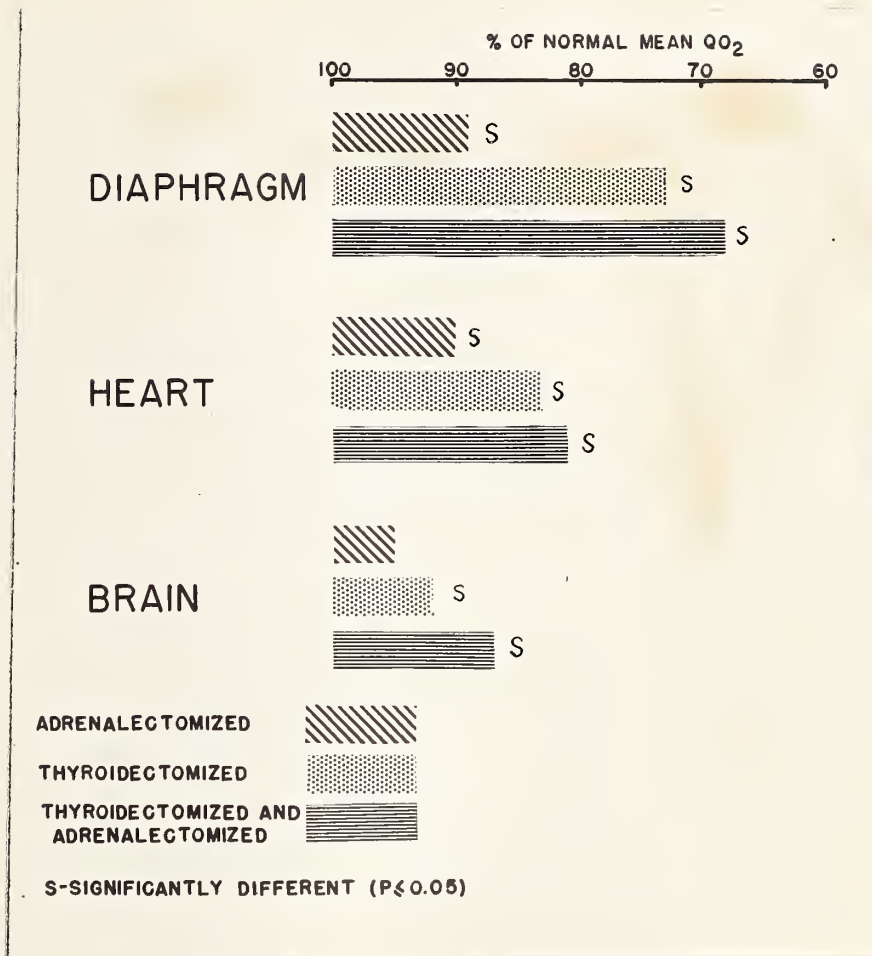


Figure II - Effect of Thyroidectomy, Adrenalectomy, and Combined Thyroidectomy and Adrenalectomy on Tissue Respiration in the Rat

hormone level is evidenced by the fact that diaphragm is depressed to the greatest extent and brain the least, with heart intermediate.

The respiration of diaphragm, heart and brain was decreased less by adrenalectomy than by thyroidectomy or by combined thyroidectomy and adrenalectomy. The respiration of all tissues was decreased more by thyroidectomy than by adrenalectomy. A greater decrease in the respiration of all tissues occurred after a combination of thyroidectomy and adrenalectomy than by either treatment alone.

Examination of Table VII reveals that administration of TSH to normal rats increased the QO_2 of diaphragm, heart and brain to above that of normal in all experiments except in the case of heart when the drug was given 24 hours before sacrificing. When TSH was administered to hypophysectomized rats the respiration of the tissues was not returned to normal levels.

VI. DISCUSSION

1. General Discussion of Tissue Respiration

A discussion of the results of tissue respiration should be prefaced by some comments on the procedure proposed by Huston and Martin. The in vitro evaluation of the pharmacological action of drugs at the cellular level after administration of the drug in vivo is complicated in standard Warburg methods by such factors as modification of the drugs and tissue metabolites by the liquid suspension medium. Cellular effects demonstrated by the addition of drugs in vitro may or may not represent the response in the intact animal, particularly in view of possible differential tissue distribution and sensitivity of the drug. Huston and Martin showed that tissue respiration can be measured with the tissues suspended in a gaseous phase of oxygen on fibre glass mats. This technique avoids variations due to different liquid media and permits quantitative assessment in vitro of the tissue effects of the drugs administered to the intact animal.

Rodnight and McIlwain (103) compared rates of respiration of brain, kidney, diaphragm and liver without added media and in olive oil, light paraffin and silicone fluid. In each case respiration rates were initially higher than those observed in saline which were run at the same time. They found that unless glucose was added the rate of respiration fell quite rapidly in brain.

Drabkin and Marsh (104) using a moist chamber respirometer on a principle similar to that of the Huston-Martin technique found that they could incubate tissue slices for as long as ten hours without appreciable diminution of the rates of oxygen uptake. They found that tissues remained viable two to three times longer than in conventional Warburg technique.

Some advantages to the technique of administering the drug to the animal and examining the tissues in oxygen would appear to be:

- (a) the drug has been administered in vivo and the distribution and response has been governed by the intact animal; adding the drug from the side arm in vitro presents a completely artificial situation;
- (b) the drug has not been diluted or extracted from the tissues by a liquid media;
- (c) variable influences due to ions or metabolites in the medium are avoided;

Disadvantages or limitations of the procedure may be summarized as follows:

- (a) once the tissue is placed on the mat further drugs and/or metabolites cannot be added to it. The technique therefore does not lend itself to an examination of substrate phenomena;
- (b) the tissue cannot act as its own control as is the case when the drug is added from a side arm. It is necessary to run control series of non-treated animals;
- (c) a not too serious disadvantage and one which is inherent in all tissue respiration studies is that once the tissue is removed from the body it progressively departs from physiological normalcy. Many factors

are involved not all of which are known. Some of the more obvious factors are loss of hormone and nervous control, limitation of supply of metabolites and ions and accumulation of metabolic end products. However, since the primary interest is the effect of the drug on the tissue at the time it is removed from the body, that is the in vivo effect, this disadvantage is not serious;

- (d) a possible disadvantage is that the tissue in contact with oxygen and not supplied with nutrient may "burn itself up." This situation would be indicated by a more rapid fall in slope of the graph and at the end of an hour the respiration rate might be expected to be below that of the tissues in fluid. However it has been found that the tissues on the mats had the least diminution of oxygen consumption (104) (93).

The above disadvantages are minimized by the procedure of extrapolation to zero time. Rate of respiration has been reduced in the cold during the preliminary manipulations and returns to a maximum at the conclusion of equilibration which is the point of extrapolation. This figure, so obtained, would appear to most closely approximate the in vivo situation.

The Huston-Martin technique was used throughout this investigation because of the advantages outlined above and because of the findings in this laboratory subsequent to those of Huston and Martin (93) (94) regarding the usefulness of this technique in pharmacological studies (95-97).

2. Tissue Respiration of Normal Animals

Examination of the normal QO_2 values from each of the series reveals a variation in the QO_2 of all tissues investigated from series to series. This variation can possibly be attributed to age and seasonal variation.

Pearce (105) found that tissue respiration in older rats was significantly less than that of younger rats. Belasco (106) found the respiration of normal kidney and liver to be a function of age. Wollenberger and Jehl (107) found that the respiration of heart decreased as mice became older.

Aron et al. (108) found that the male rat reveals a fluctuation in thyroid activity at various times of the year and Bernstein (109) found a normal seasonal variation in epithelial cell height in the thyroid gland. This seasonal variation cannot apparently be correlated with environmental temperature.

These factors along with others not so apparent could possibly be the cause of the fluctuation in the QO_2 values of the normal animals. These findings illustrate the necessity of investigating control animals at the time an experiment is undertaken.

3. Effect of Thyroidectomy on the Respiration of Diaphragm and Heart

The respiration of diaphragm was decreased by thyroidectomy in Series I, II, and III. The decrease was significant in Series II and III. It has been generally agreed that thyroidectomy causes the QO_2 of the diaphragm to be depressed significantly (56) (60) (61) (64).

Thyroidectomy depressed the respiration of the heart in Series I, II, and III. There was a significant depression in the last two series. Saunders et al. (68) found that thyroidectomy did not produce a significant decrease in the respiration of the heart. However Barker and Klitgaard (61) and Whaley et al. (64) observed a significant depression in the respiration of the heart after thyroidectomy.

4. Effect of Triiodothyronine on Tissue Respiration

a. Diaphragm

The rats of Series I and III were injected 4 days before sacrificing whereas the rats of Series II were injected 7 days before sacrificing. Treatment of normal rats with triiodothyronine produced an increase in the respiration of the diaphragm in Series I, II, and III, but the increase was not significant in Series II. This indicates that injection of triiodothyronine four days before sacrificing is closer to the optimum peak of activity of triiodothyronine than is seven days. This is similar to the findings of Whaley et al. (64) who found that the diaphragm from thyroidectomized rats reached its peak rate of respiration four days after a single injection of triiodothyronine.

Treatment of thyroidectomized rats with triiodothyronine resulted in a significant increase in the respiration of the diaphragm in all three series. This is in agreement with studies carried out by other workers (63) (64) (44).

b. Heart

Treatment of normal rats with triiodothyronine increased the respiration of heart, but not significantly, in Series I, II, and III. Similar studies by other workers have not been carried out with triiodothyronine on normal rats.

Treatment of thyroidectomized animals with triiodothyronine increased the respiration of heart in all cases but a significant increase occurred only in the second series. The increase was approaching

significance in the third series. Most workers have found that triiodo-thyronine administration increases the respiration of heart significantly (63) (64) (68) (51).

5. Effect of the Special Diet on Tissue Respiration

Leblond and Eartly (102) reported that if thyroidectomized rats were fed a diet which was low in iodine and deficient in animal protein, their body weight was reduced compared to those thyroidectomized rats which received Purina Fox Chow. These rats also showed symptoms which were quite comparable to the classical descriptions of athyroidism. These workers suggested that there were extrathyroidal areas in the rat which converted iodine and tyrosine into the thyroid hormones. They also suggested that animal protein would contain appreciable amounts of thyroxine. Similar suggestions of extrathyroidal activity have been made by Morton et al. (110).

When the results of Series II and Series III (special diet) are examined it is found that normal QO_2 values from either series are closely related. Thyroidectomy decreased the respiration of the diaphragm 32% below that of normal in Series II and only 16% below that of normal in the animals on the special diet. Thyroidectomy depressed the heart respiration 7% in Series II and 10% in the animals maintained on the special diet. It appears, then, on the basis of depression of respiration of diaphragm and heart that the special diet did not affect the respiration of these two tissues appreciably in thyroidectomized and normal rats. These findings would agree with the work of Taurog et al. (111) who failed to find any significant extrathyroidal hormone formation in rats which had been thyroidectomized and fed on a diet with a moderate iodine intake.

6. Effect of Thyroidectomy and Triiodothyronine on Tissue Respiration of the Brain

Thyroidectomy significantly lowered the respiration of the brain. Triiodothyronine given to normal and thyroidectomized rats significantly increased the respiration of the brain.

Retarded mental activity is an accompanying symptom of human cretinism. Brain respiration therefore has often been investigated in altered thyroid states. However the majority of workers have found that the respiration of the brain is surprisingly not significantly influenced by thyroidectomy or administration of thyroid hormones (61) (62) (63) (69) (58) (70) (70a) (71). On the other hand Cohen and Gerard (72) and Rossiter (73) have found administration of thyroid hormone results in an increase of brain respiration.

Although in our results the percentage decrease and increase in the brain QO_2 are not large after thyroidectomy and triiodothyronine administration respectively, a significant alteration in the respiration of the brain was detected by the Huston-Martin technique after these treatments.

7. Effect of Thyroidectomy, Adrenalectomy, and Combined Thyroidectomy and Adrenalectomy on Tissue Respiration

Adrenalectomy depressed the respiration of diaphragm, heart, and brain; the diaphragm and heart QO_2 being lowered by a significant amount. Thyroidectomy depressed the respiration of all three tissues significantly below that of normal. A combination of thyroidectomy and adrenalectomy caused the QO_2 of all three tissues to drop significantly below that of normal. This combined surgery caused a significant decrease in the QO_2 below that of all tissues from adrenalectomized animals but not below that of tissues from thyroidectomized animals.

The depression caused by thyroidectomy has already been discussed earlier. An explanation of the results obtained after adrenalectomy is difficult because of the many hormones affected by adrenalectomy. It has been generally agreed that cortisone depresses the QO_2 of excised tissues (77) (112) (113). Cortisone, however, has been reported to influence different tissues in different ways in both sexes. Lacroix and Leusen (78) found that the QO_2 of heart was markedly depressed after cortisone treatment while that of the diaphragm was increased in male rats. Lacroix (114) also found that administration of cortisone to male thyroidectomized rats did not significantly influence the effect of thyroidectomy on myocardium but returned the respiration of diaphragm to normal.

Adrenaline, on the other hand, enhances the action of thyroid hormone. Swanson (79) found that thyroidectomy inhibited and thyroxine potentiated the calorogenic effect of adrenaline. Eskelson (80) and Botkin and Jensen (115) have found that adrenaline reduced the gland content and blood level of I^{131} . This response to adrenaline is interpreted as being due to the increased utilization of circulating thyroid hormone caused by adrenaline.

It appears, then, that thyroid hormone action is enhanced by adrenaline and antagonized by cortisone although the action of cortisone is not the same for all tissues and it seems to be sex specific in some tissues. The results obtained in our study agree with the BMR studies of Hoffmann et al. (74) who found that total body oxygen consumption was decreased 10% after adrenalectomy and 20% after adrenalectomy and thyroidectomy combined. He also found that adrenal cortical hormone raised the BMR

of thyroidectomized-adrenalectomized rats.

Explanation of the results obtained is difficult because of the many hormones elaborated by the adrenal gland and the interactions which occur between adrenal hormones and other glands such as the pituitary and the gonads.

8. Effect of Hypophysectomy and Thyrotropin (TSH) on Tissue Respiration

In the preliminary series (Series VI) hypophysectomy caused a depression of respiration in diaphragm, heart, and brain; the depression being significant in the last two tissues. In Series VII, a significant depression of respiration in the diaphragm was produced by hypophysectomy but the depression was not significant in the other two tissues. Although the depression is not significant in all cases, all tissues are depressed by hypophysectomy and the lack of significance could possibly be due to the small number of animals used. Other workers have found that hypophysectomy does cause a reduction of respiration at the tissue level (89, 90).

Treatment of normal animals with TSH four hours before sacrificing caused an increase in the respiration of diaphragm, heart and brain. A significant increase was realized only in brain. These results are in agreement with those of Jandorf and Williams (89) who found that the QO_2 of liver and diaphragm was increased with TSH administration. The administration of TSH to hypophysectomized rats by MacLeod and Reiss (88) increased the oxygen consumption of brain.

Treatment of normal animals with TSH twenty-four hours before sacrificing caused no change in the respiration of diaphragm, a decrease

in the respiration of the heart and an increase in the respiration of the brain. Neither the increase nor the decrease in respiration was significant.

Administration of TSH to hypophysectomized rats four hours before sacrificing caused no change in the respiration of the diaphragm a depression in the respiration of the heart, and a slight increase in the respiration of the brain. Administration of TSH twenty-four hours before sacrificing resulted in essentially the same results as administration four hours before sacrificing with the exception that the brain respiration was depressed.

Brown-Grant et al. (116) injected TSH into normal rabbits and found that the period of accelerated I^{131} release caused by TSH was followed by a period when the output of thyroid I^{131} was completely inhibited. He felt that the explanation for the inhibition was that exogenous TSH would suppress endogenous TSH which would in turn depress thyroid activity. If this is true it would appear that in those rats where TSH caused a depression of respiration, the peak of TSH activity may have occurred before the time the animals were sacrificed.

VII. SUMMARY AND CONCLUSIONS

The aim of this research was to determine the effects of altered thyroid states on tissue respiration in the rat.

1. It was found that thyroidectomy significantly depressed the respiration of diaphragm, heart and brain, except in the first series.
2. Treatment of normal rats with triiodothyronine increased the respiration of the diaphragm significantly when the drug was administered four days before sacrificing. When the drug was administered seven days before sacrificing there was an increase in respiration but it was not significant. Treatment of thyroidectomized rats with triiodothyronine increased the respiration of the diaphragm significantly in all cases.
3. Administration of triiodothyronine to normal rats increased the respiration of the heart but not significantly. When the drug was given to thyroidectomized rats an increase in the heart QO_2 occurred in all cases. The increase was significant in the seven day rats. The increase was approaching significance in one series of four day rats and it was not significant in the other.
4. The special diet appeared to make no appreciable difference in the QO_2 of the diaphragm and heart.
5. Administration of triiodothyronine to normal and thyroidectomized rats increased the QO_2 of the brain significantly.
6. Adrenalectomy depressed the respiration of the diaphragm, heart

and brain; the diaphragm and heart QO_2 being lowered significantly. A combination of thyroidectomy and adrenalectomy caused the QO_2 of all tissues to drop significantly below that of normal. This combined surgery caused a significant decrease in the QO_2 of all three tissues below that of adrenalectomized animals. The respiration of tissues from animals which had been both thyroidectomized and adrenalectomized was not below that of tissues from thyroidectomized animals.

7. Hypophysectomy depressed the respiration of diaphragm, heart and brain; the depression being significant in the heart and brain in the preliminary series and in the diaphragm in the last series.
8. Administration of TSH to normal rats four hours before sacrificing caused an increase in the respiration of the diaphragm, heart and brain. A significant increase was realized only in the brain. Treatment of normal animals with TSH twenty-four hours before sacrificing caused no change in the respiration of the diaphragm, a reduced respiration in the heart, and an increase in respiration in the brain. Administration of TSH to hypophysectomized rats four hours before sacrificing caused no change in the respiration of the diaphragm, a depression in the respiration of the heart and a slight increase in the respiration of the brain.
Administration of TSH to hypophysectomized rats twenty-four hours before sacrificing resulted in essentially the same results as administration of the drug four hours before sacrificing with the exception that the brain respiration was depressed.
9. The significance of the findings is discussed.

The first of these is the fact that the
government has been unable to
bring about a general agreement
among the various interest groups
concerned with the problem of
the distribution of income. This
is due to the fact that each group
has its own special interests and
is not willing to sacrifice them for
the good of the community as a whole.

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TABLE i

SERIES I

RESPIRATION OF DIAPHRAGM TISSUE FROM NORMAL, NORMAL-TREATED,
THYROIDECTOMIZED, AND THYROIDECTOMIZED-TREATED RATS

Figures represent QO_2 values in ml O_2 per gram wet weight per hour.

The treated animals were injected with L-triiodothyronine (T_3) 4 days before sacrificing.

NORMAL		THYROIDECTOMIZED	
	+ T_3		+ T_3
1.08	1.38	1.95	1.62
1.22	1.12	1.52	1.64
1.26.	0.91	1.80	1.77
1.07	0.96	1.35	1.35
1.94	1.42	1.28	1.46
1.06	2.50	1.45	1.60
1.34.	2.18	1.35	1.78
1.80	1.56	1.37	2.21
1.70	1.85	1.20	2.28
1.35	1.83	1.17	2.28
1.40	1.52	1.24	2.38
1.23	2.13	1.90	2.13
1.26	2.20	1.95	1.92
1.20	2.15	1.99	1.89
1.50	1.12	1.15	1.54
1.30	1.67	1.12	1.35
2.01	1.63	1.45	1.34
2.07	0.57	1.10	2.02
1.03	1.73	1.15	2.49
1.60	1.71	1.36	1.35
1.32	1.55	1.10	1.16
1.12	1.95	1.04	1.45
1.42	1.33	1.08	1.37

TABLE i--Continued

	NORMAL	NORMAL +T ₃	THYROIDECTOMIZED	THYROIDECTOMIZED +T ₃
	1.52	1.56	1.16	1.14
	1.25	2.01	0.94	1.06
	1.37	1.65	1.17	
	1.50	2.46	1.00	
	2.47	1.55	1.10	
	1.93	1.69	0.77	
	2.04	1.55	1.18	
	1.50	1.73	1.40	
	1.60	1.60	1.29	
	1.70	1.80		
	1.80	2.05		
	1.27	1.67		
	1.27	1.89		
	1.13	2.60		
	1.02	2.10		
	0.95			
Mean QO ₂	1.45	1.71	1.32	1.70
s. d. m.	0.35	0.42	0.30	0.41

TABLE ii

SERIES I

RESPIRATION OF HEART SLICES FROM NORMAL, NORMAL-TREATED,
THYROIDECTOMIZED, AND THYROIDECTOMIZED-TREATED RATS

Figures represent QO_2 values in ml O_2 per gram wet weight per hour.

The treated animals were injected with L-triiodothyronine (T_3) 4 days before sacrificing.

NORMAL		THYROIDECTOMIZED	
	+ T_3		+ T_3
2.97	1.38	2.50	2.20
2.50	2.29	2.70	2.22
2.69	2.17	3.17	1.75
2.15	2.60	2.10	2.76
2.12	2.85	2.03	2.62
1.88	2.35	2.07	2.46
1.78	2.43	2.36	1.80
2.20	2.30	2.13	2.59
2.29	2.55	2.30	2.34
1.90	2.27	2.32	2.50
2.20	2.87	2.88	2.45
2.13	2.36	2.50	2.27
2.32	2.20	2.93	2.44
2.89	2.75	2.40	2.65
2.84	1.95	2.68	2.93
2.45	2.35	2.28	2.58
2.88	2.20	2.31	2.70
2.60	2.84	2.10	2.29
2.70	2.57	2.37	2.42
2.52	2.37	2.43	2.06
2.42	2.88	2.57	2.40
1.62	2.85	2.24	2.75
2.07	3.10	2.30	2.97

TABLE ii--Continued

	NORMAL	NORMAL +T ₃	THYROIDECTOMIZED	THYROIDECTOMIZED +T ₃
	2.06	2.83	2.20	2.98
	2.36	2.30	2.64	2.67
	2.37	2.85	2.37	2.54
	1.85	2.70	2.55	3.00
	2.02	2.65	2.29	2.50
	2.50	2.53	2.69	2.65
	2.35	2.50	2.22	2.33
	2.78	3.05	2.77	2.44
	2.68	2.99	2.39	2.35
	2.20	2.32		2.80
	2.98	2.62		
	2.08	2.69		
	3.15	2.85		
	2.64			
	2.85			
	2.57			
	2.60			
	2.62			
	2.85			
Mean QO ₂	2.42	2.54	2.43	2.50
s. d. m.	0.36	0.32	0.27	0.27

TABLE iii

SERIES II

RESPIRATION OF DIAPHRAGM TISSUE FROM NORMAL, NORMAL-TREATED,
THYROIDECTOMIZED, AND THYROIDECTOMIZED-TREATED RATS

Figures represent QO_2 values in ml O_2 per gram wet weight per hour.

The treated animals were injected with L-triiodothyronine (T_3) 7 days before sacrificing.

NORMAL		THYROIDECTOMIZED	
	+ T_3		+ T_3
1.99	1.50	1.64	1.98
1.70	2.28	1.12	2.26
1.58	1.84	1.19	2.33
2.19	1.57	1.17	2.01
2.30	1.58	1.62	2.51
2.03	2.31	1.17	1.96
1.73	1.97	1.13	2.42
1.89	1.94	1.12	2.80
1.32	2.25	1.15	2.67
1.65	2.11	1.50	2.39
1.41	1.57	1.25	1.70
2.07	2.19	1.55	2.00
2.12	1.90	1.12	2.73
2.15	1.53	1.10	2.14
1.45	1.22	1.22	1.75
1.16	2.11	1.10	1.81
1.60	1.25	1.17	1.50
1.54	2.12	0.94	2.32
1.43	1.38	0.96	1.52
2.02	1.90	0.81	0.98
1.40	2.26	1.06	0.93
1.26	2.20	0.96	1.67
1.59	1.72	1.09	1.68
1.33		1.21	1.37

TABLE iii--Continued

	NORMAL	NORMAL +T ₃	THYROIDECTOMIZED	THYROIDECTOMIZED +T ₃
	1.64		1.04	1.19
	1.95		0.95	1.46
	1.86		1.26	1.36
			0.96	
Mean QO ₂	1.72	1.86	1.16	1.91
s. d. m.	0.31	0.34	0.31	0.51

TABLE iv

SERIES II

RESPIRATION OF HEART SLICES FROM NORMAL, NORMAL-TREATED,
THYROIDECTOMIZED, AND THYROIDECTOMIZED-TREATED RATS

Figures represent QO_2 values in ml O_2 per gram wet weight per hour.

The treated animals were injected with L-triiodothyronine (T_3) 7 days before sacrificing.

NORMAL		NORMAL THYROIDECTOMIZED		THYROIDECTOMIZED	
		+T ₃		+T ₃	
2.95	2.91	2.54	2.51		
2.66	2.90	2.73	2.63		
2.78	2.87	2.65	2.65		
2.35	3.03	2.59	2.73		
2.78	2.68	2.45	2.45		
2.69	2.69	2.30	3.10		
2.27	2.73	2.55	3.00		
2.45	2.62	2.28	3.00		
2.30	2.34	2.22	3.22		
3.10	3.15	1.76	2.91		
2.81	3.30	2.37	2.74		
3.01	3.89	2.50	2.55		
2.62	2.90	2.12	2.83		
2.29	3.36	2.45	2.52		
2.34	2.60	2.32	2.88		
2.75	2.90	2.29	2.60		
2.61	2.57	2.05	2.55		
2.50	2.38	2.10	2.50		
2.40	2.56	2.70	2.68		
2.72	2.95	2.43	2.82		
2.82	3.06	2.70	3.01		
3.02	2.57	2.49	2.95		
2.89	2.44	2.99	3.15		
3.02	3.00	2.80	2.75		
2.49	2.60	2.55	2.73		

TABLE iv--Continued

	NORMAL	NORMAL +T ₃	THYROIDECTOMIZED	THYROIDECTOMIZED +T ₃
	2.55	2.36	2.32	3.35
	2.50	2.70	2.71	
	2.53	2.49	2.71	
	2.50	2.43	2.80	
	3.15	2.68	2.55	
			2.61	
			2.40	
Mean QO ₂	2.67	2.79	2.47	2.73
s. d. m.	0.26	0.34	0.25	0.41

TABLE v

SERIES III

RESPIRATION OF DIAPHRAGM TISSUE FROM NORMAL, NORMAL-TREATED,
THYROIDECTOMIZED, AND THYROIDECTOMIZED-TREATED RATS

Figures represent QO_2 values in ml O_2 per gram wet weight per hour.

The rats were fed on a special diet. The treated rats were injected with L-triiodothyronine (T_3) 4 days before sacrificing.

NORMAL		THYROIDECTOMIZED	
	+ T_3		+ T_3
1.80	2.08	1.23	2.40
1.38	2.09	1.19	1.90
1.46	2.46	1.51	1.95
1.01	2.28	1.30	2.12
1.22	2.17	1.14	1.66
1.31	2.44	1.35	2.14
1.79	2.30	1.00	1.87
1.55	2.38	1.12	1.70
2.14	2.80	1.09	1.88
1.71	2.04	1.50	2.19
1.80	1.94	1.80	2.31
2.12	2.51	1.71	1.83
1.29	2.14	1.35	1.91
1.34	1.95	1.38	2.14
1.62	1.88	1.35	2.09
1.84	1.93	1.83	1.30
2.25	2.05	1.60	1.18
1.92	1.90	1.92	1.54
1.41	1.60	1.35	2.05
1.45	1.47	1.40	1.85
1.43	1.70	1.45	
1.83	2.02	1.55	
2.15	1.70	1.41	
1.98	2.09	1.52	

TABLE v--Continued

	NORMAL	NORMAL	THYROIDECTOMIZED	THYROIDECTOMIZED
		+T ₃		+T ₃
	2.40	2.35	1.18	
	2.38	1.93	1.35	
	2.31		1.32	
	1.76			
	1.43			
Mean QO ₂	1.67	2.08	1.40	1.90
s.d.m.	0.48	0.30	0.23	0.29

TABLE vi

SERIES III

RESPIRATION OF HEART SLICES FROM NORMAL, NORMAL-TREATED,
THYROIDECTOMIZED, AND THYROIDECTOMIZED-TREATED RATS

Figures represent QO_2 values in ml O_2 per gram wet weight per hour.

The rats were fed on a special diet. The treated rats were injected with L-triiodothyronine (T_3) 4 days before sacrificing.

NORMAL		THYROIDECTOMIZED	
	+ T_3		+ T_3
2.68	2.63	2.15	2.76
2.64	2.46	2.33	2.81
2.68	2.89	2.47	3.10
2.90	2.85	2.70	2.61
2.15	3.04	2.65	2.79
2.43	3.17	2.84	2.35
2.94	2.65	2.19	1.92
2.96	2.82	1.97	2.45
2.70	3.21	2.01	1.80
2.93	2.94	2.37	2.62
2.28	2.31	2.31	2.68
2.84	2.86	2.55	2.75
2.58	2.55	2.51	3.04
2.61	2.85	2.81	2.99
2.57	2.80	2.26	2.90
3.15	3.33	2.44	2.40
2.74	3.05	2.85	2.52
3.10	2.21	2.80	2.65
2.78	2.65	2.44	2.80
3.00	2.88	2.31	2.74
2.64	2.72	2.75	2.66
3.30	2.47	2.18	2.60
2.94	2.88	2.45	2.25

TABLE vii

SERIES IV

RESPIRATION OF BRAIN SLICES FROM NORMAL, NORMAL-TREATED,
THYROIDECTOMIZED, AND THYROIDECTOMIZED-TREATED RATS

Figures represent $\dot{Q}O_2$ values in ml O_2 per gram wet weight per hour.

The treated animals were injected with L-triiodothyronine (T_3) 4 days before sacrificing.

NORMAL		THYROIDECTOMIZED	
	+ T_3		+ T_3
3.10	3.00	2.89	2.96
2.85	3.74	2.77	2.95
2.99	3.12	3.18	3.12
3.35	3.13	2.98	3.13
2.97	3.15	2.55	3.09
2.73	2.89	2.27	3.13
2.77	3.22	2.53	2.86
2.89	3.02	2.48	2.95
3.11	2.80	2.55	2.43
2.75	2.89	2.33	2.42
2.78	2.99	2.75	2.26
2.98	2.77	2.67	2.41
3.17	2.97	2.54	3.40
2.67	2.84	2.65	2.96
2.63	2.54	2.67	3.17
2.35	2.28	2.68	2.82
3.17	3.00	2.45	2.57
2.67	2.65	2.44	2.43
2.63	2.68	2.74	2.91
2.35	3.06	2.53	3.09
3.00	2.58	2.32	2.79
2.65	2.52	2.55	2.85
2.60	2.74	2.49	2.47

TABLE vii--Continued

	NORMAL	NORMAL +T ₃	THYROIDECTOMIZED	THYROIDECTOMIZED +T ₃
	3.23	3.10	2.39	2.46
	2.60	3.15	2.90	2.31
	2.55	3.24	2.84	2.27
	2.74	2.90	2.66	3.11
	2.51	3.00	2.86	2.95
	2.63	2.80	3.00	2.74
	2.83	2.69	2.80	2.87
	2.57	3.01	2.78	3.12
	3.32	2.90	2.49	2.95
	2.89	3.04	2.96	2.81
	2.96	3.34	2.64	2.68
	2.85	2.95	2.62	2.95
	3.20		2.75	2.77
	2.70		3.04	3.11
	2.54		2.84	
	2.46		2.47	
	2.82			
	2.45			
Mean QO ₂	2.81	2.93	2.67	2.82
s. d. m.	0.26	0.26	0.22	0.29

TABLE viii

SERIES V

RESPIRATION OF DIAPHRAGM TISSUE FROM NORMAL,
ADRENALECTOMIZED, THYROIDECTOMIZED AND
THYROIDECTOMIZED-ADRENALECTOMIZED RATS

Figures represent QO_2 values in ml O_2 per gram wet weight per hour.

	NORMAL	ADRENALECTO- MIZED	THYROIDECTO- MIZED	THYROIDECTOMIZED AND ADRENALECTOMIZED
	1.72	1.45	1.45	1.44
	1.78	1.40	1.46	1.16
	1.64	1.24	1.30	1.23
	1.81	1.16	1.45	1.13
	1.60	1.67	1.21	1.25
	1.73	1.50	1.25	1.22
	1.52	1.31	1.18	1.35
	1.32	1.25	0.96	1.25
	1.87	1.55	1.39	1.21
	2.25	1.13	1.46	1.42
	1.88	1.97	1.12	1.71
	1.75	1.85	1.31	1.56
	2.11	1.45	1.41	1.15
	2.17	1.41	1.77	1.06
	1.41	1.93	1.09	1.60
	1.46	1.99	1.13	1.41
	1.50	2.42	1.14	1.25
	1.42	2.05	1.39	1.06
	2.10	1.83	1.71	1.11
	1.92	1.85		1.21
	2.61			
	2.53			
Mean QO_2	1.82	1.62	1.33	1.24
s. d. m.	0.35	0.34	0.20	0.18

TABLE ix

SERIES V

RESPIRATION OF HEART SLICES FROM NORMAL,
ADRENALECTOMIZED, THYROIDECTOMIZED AND
THYROIDECTOMIZED-ADRENALECTOMIZED RATS

Figures represent QO_2 values in ml O_2 per gram wet weight per hour.

NORMAL ADRENALECTO- MIZED		THYROIDECTO- MIZED	THYROIDECTOMIZED AND ADRENALECTOMIZED
2.54	2.19	2.93	1.98
2.94	2.70	2.96	2.15
2.64	2.50	2.68	2.24
2.80	2.50	2.70	2.41
2.60	2.19	2.15	2.50
2.83	2.36	2.35	2.53
2.82	1.86	2.13	3.03
2.78	2.05	2.55	2.38
2.69	2.79	1.46	2.77
2.75	2.71	1.64	2.17
2.46	2.79	2.28	2.38
2.91	2.90	2.41	2.07
2.61	2.66	2.22	2.27
2.97	2.69	2.49	2.03
3.11	2.36	1.96	2.01
3.16	2.66	1.98	2.11
2.58	2.62	1.97	2.29
2.93	2.57	2.48	2.16
	2.13	2.15	2.09
	2.77	2.67	2.34
			1.84
			1.97
			1.92
Mean QO_2	2.78	2.31	2.25
s. d. m.	0.19	0.39	0.27

TABLE x
SERIES V

RESPIRATION OF BRAIN SLICES FROM NORMAL,
ADRENALECTOMIZED, THYROIDECTOMIZED AND
THYROIDECTOMIZED-ADRENALECTOMIZED RATS

Figures represent QO_2 values in ml O_2 per gram wet weight per hour.

NORMAL ADRENALECTO- THYROIDECTO- THYROIDECTOMIZED			
	MIZED	MIZED	AND ADRENALECTOMIZED
2.97	3.00	3.60	2.85
3.22	3.00	3.04	2.97
2.76	3.17	3.60	2.90
3.40	3.07	3.17	2.82
3.35	2.83	3.06	2.90
3.20	2.82	2.84	2.95
3.40	2.75	2.55	2.95
3.25	2.70	2.78	2.61
3.05	3.10	2.32	3.08
3.24	2.75	2.13	2.99
3.27	4.40	3.46	2.77
3.40	4.25	3.35	2.69
3.40	3.02	3.13	2.75
3.40	3.38	3.25	2.90
3.40	3.31	2.23	2.90
3.10	2.61	2.59	2.88
3.05	2.85	2.94	2.80
2.94	2.77	3.60	2.82
2.97	3.05	3.18	2.88
3.80	2.80		2.75
3.37			2.43
			2.41
			2.34
			2.69
			2.80
			3.27
Mean QO_2	3.24	2.99	2.81
s. d. m.	0.22	0.49	0.20

TABLE xi

SERIES VI

RESPIRATION OF DIAPHRAGM, HEART, AND BRAIN SLICES FROM
NORMAL RATS

Figures represent QO_2 values in ml O_2 per gram wet weight per hour.

	DIAPHRAGM	HEART	BRAIN
	1.30	2.46	3.05
	1.70	2.49	3.50
	1.95	2.21	2.35
	2.01	2.42	2.80
	1.90	2.70	2.87
	1.47	2.74	2.80
	1.63	2.76	2.83
	1.96	2.58	2.70
	1.95	2.54	2.63
	2.00	2.41	2.71
		2.27	2.65
		2.57	2.75
Mean QO_2	1.79	2.51	2.80
s. d. m.	0.24	0.17	0.26

TABLE xii

SERIES VI

RESPIRATION OF DIAPHRAGM, HEART, AND BRAIN SLICES FROM
HYPOPHYSECTOMIZED RATS

Figures represent QO_2 values in ml O_2 per gram wet weight per hour.

	DIAPHRAGM	HEART	BRAIN
	1.20	2.45	2.45
	1.98	2.22	3.08
	1.40	2.17	2.90
	1.09	2.18	2.75
	1.87	2.28	2.41
	1.82	2.25	2.45
	1.72	1.98	2.75
	1.63	2.28	2.80
	2.01	2.34	2.40
	1.68	2.38	2.85
	1.11	1.80	2.00
	1.49	2.13	2.21
	1.37	2.10	2.17
		2.15	2.20
Mean QO_2	1.57	2.19	2.53
s. d. m.	0.30	0.16	0.31

TABLE xiii

SERIES VII

RESPIRATION OF DIAPHRAGM TISSUE FROM NORMAL, NORMAL-TREATED,
HYPOPHYSECTOMIZED, AND HYPOPHYSECTOMIZED-TREATED RATS

Figures represent QO_2 values in ml O_2 per gram wet weight per hour.

The treated animals were injected with thyrotropin (TSH) 4 hours and 24 hours before sacrificing as indicated.

	NORMAL	NORMAL +TSH (4 hrs.)	NORMAL +TSH (24 hrs.)	HYPOPHY- SECTO- MIZED	HYPOPHY- SECTO- MIZED +TSH (4 hrs.)	HYPOPHY- SECTO- MIZED +TSH (24 hrs.)
	1.93	1.97	2.05	1.40	1.91	1.46
	1.54	2.18	1.85	1.15	2.12	1.36
	2.23	2.42	2.00	1.89		1.41
	1.89	2.55	2.13	2.12		1.54
	2.47	2.12	1.90	1.63		2.00
	2.28	2.00	2.53	1.61		1.79
	2.25	2.60			1.92	1.99
	1.94	2.55			1.51	
Mean QO_2	2.07	2.30	2.08	1.63	1.63	1.65
s. d. m.	0.28	0.24	0.22	0.31	0.23	0.24

TABLE xiv

SERIES VII

RESPIRATION OF HEART SLICES FROM NORMAL, NORMAL-TREATED,
HYPOPHYSECTOMIZED, AND HYPOPHYSECTOMIZED-TREATED RATS

Figures represent QO_2 values in ml O_2 per gram wet weight per hour.

The treated animals were injected with thyrotropin (TSH) 4 hours and 24 hours before sacrificing as indicated.

	NORMAL	NORMAL +TSH (4 hrs.)	NORMAL +TSH (24 hrs.)	HYPOPHY- SECTO- MIZED	HYPOPHY- SECTO- MIZED +TSH (4 hrs.)	HYPOPHY- SECTO- MIZED +TSH (24 hrs.)
	2.64	3.05	2.91	2.34	2.82	2.25
	2.77	2.58	2.77	2.86	2.90	2.63
	2.57	3.25	2.98	2.20	2.70	2.70
	2.90	3.22	2.64	2.54	2.92	2.38
	3.11	2.77	2.70	2.82		2.60
	3.18	2.92	2.62	2.86		2.36
	2.81	3.16	2.84			2.49
	3.10	2.95				
Mean QO_2	2.89	2.99	2.78	2.60	2.41	2.49
s. d. m.	0.21	0.22	0.13	0.27	0.21	0.34

TABLE
I

Summary of the results of the experiments on the effect of the concentration of the solution on the rate of the reaction

Concentration of the solution (M)

Rate of the reaction (M/min)

Concentration of the solution (M)	Rate of the reaction (M/min)
0.01	0.001
0.02	0.002
0.03	0.003
0.04	0.004
0.05	0.005
0.06	0.006
0.07	0.007
0.08	0.008
0.09	0.009
0.10	0.010
0.11	0.011
0.12	0.012
0.13	0.013
0.14	0.014
0.15	0.015
0.16	0.016
0.17	0.017
0.18	0.018
0.19	0.019
0.20	0.020
0.21	0.021
0.22	0.022
0.23	0.023
0.24	0.024
0.25	0.025
0.26	0.026
0.27	0.027
0.28	0.028
0.29	0.029
0.30	0.030
0.31	0.031
0.32	0.032
0.33	0.033
0.34	0.034
0.35	0.035
0.36	0.036
0.37	0.037
0.38	0.038
0.39	0.039
0.40	0.040
0.41	0.041
0.42	0.042
0.43	0.043
0.44	0.044
0.45	0.045
0.46	0.046
0.47	0.047
0.48	0.048
0.49	0.049
0.50	0.050
0.51	0.051
0.52	0.052
0.53	0.053
0.54	0.054
0.55	0.055
0.56	0.056
0.57	0.057
0.58	0.058
0.59	0.059
0.60	0.060
0.61	0.061
0.62	0.062
0.63	0.063
0.64	0.064
0.65	0.065
0.66	0.066
0.67	0.067
0.68	0.068
0.69	0.069
0.70	0.070
0.71	0.071
0.72	0.072
0.73	0.073
0.74	0.074
0.75	0.075
0.76	0.076
0.77	0.077
0.78	0.078
0.79	0.079
0.80	0.080
0.81	0.081
0.82	0.082
0.83	0.083
0.84	0.084
0.85	0.085
0.86	0.086
0.87	0.087
0.88	0.088
0.89	0.089
0.90	0.090
0.91	0.091
0.92	0.092
0.93	0.093
0.94	0.094
0.95	0.095
0.96	0.096
0.97	0.097
0.98	0.098
0.99	0.099
1.00	0.100

TABLE xv

SERIES VII

RESPIRATION OF BRAIN SLICES FROM NORMAL, NORMAL-TREATED,
HYPOPHYSECTOMIZED, AND HYPOPHYSECTOMIZED-TREATED RATS

Figures represent QO_2 values in ml O_2 per gram wet weight per hour.

The treated animals were injected with thyrotropin (TSH) 4 hours and 24 hours before sacrificing as indicated.

	NORMAL	NORMAL +TSH (4 hrs.)	NORMAL +TSH (24 hrs.)	HYPOPHY- SECTO- MIZED	HYPOPHY- SECTO- MIZED +TSH (4 hrs.)	HYPOPHY- SECTO- MIZED +TSH (24 hrs.)
	2.94	3.09	3.28	3.20	2.65	2.40
	2.70	3.00	3.10	2.82	2.54	2.54
	3.15	3.60	3.31	2.54	2.95	2.30
	3.21	3.47	3.37	2.54	3.00	2.40
	2.83	2.81	2.67	2.51		2.51
	2.74	2.85	2.77	2.75		2.35
	2.80	3.25				2.56
	2.57	3.50				
Mean QO_2	2.87	3.20	3.08	2.73	2.79	2.44
s.d.m.	0.21	0.29	0.27	0.24	0.19	0.09

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